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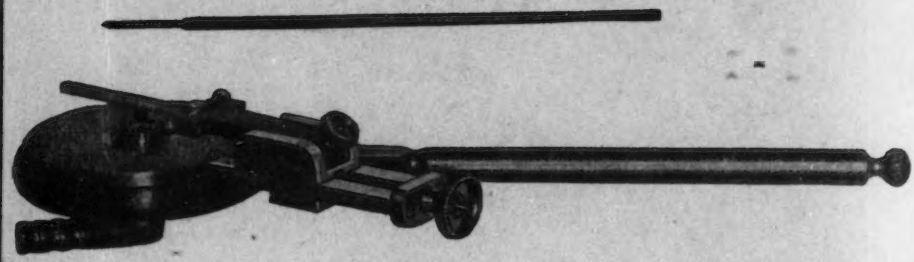

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THE INFLUENCE OF UREA UPON BLOOD CLOTTING

II. Tissue Fibrinogen Clotting and Tissue Fibrinogen

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In an article recently published (1), we concluded, as the result of a study of the action of urea upon thrombin clotting, that at least three effects can be produced by the addition of urea to clotting systems. In low concentrations urea accelerates clotting by liberation of reserve supplies of thrombin precursors, i.e., cephalin and prothrombin. In higher concentrations (up to about 5 grams per 100 cc.) it retards clotting by delaying the union of thrombin with blood fibrinogen to form fibrin. In still higher concentrations (8 grams, or more, per 100 cc.) it delays, or completely inhibits clotting by peptising the fibrin and so preventing its orientation into the solid structure of the clot. All these processes may go on at the same time. The resultant effect is shown by the change in clotting time from that of the control.

In the first part of this article we shall record the results of a similar study on tissue fibrinogen clotting. According to the theory of Mills and Mathews (2) tissue fibrinogen clotting is a process distinct from thrombin clotting and is due to the union of tissue fibrinogen (a globulin-cephalin complex . . . Mills, (3)) through calcium, with blood fibrinogen to form tissue fibrinogen fibrin. The immediate difference between thrombin and tissue fibrinogen clotting is, therefore, that thrombin unites directly with blood fibrinogen whereas tissue fibrinogen requires calcium to link with blood fibrinogen. Calcium is, of course, necessary for thrombin clotting, but in this case for the formation of thrombin, by linking together cephalin and prothrombin.

In the second part of this paper we shall discuss the action of urea upon tissue fibrinogen itself. Such a direct study of thrombin is not yet possible, since that clotting factor cannot be prepared in a stable condition.

I. The influence of urea upon tissue fibrinogen clotting. We

used, in this study, citrated horse plasma which had been deprived of platelets and tissue fibrinogen by centrifuging for 2 hours and then filtering through a Berkefeld N filter. In the following tables the term relative clotting time refers to the ratio.

$$\frac{\text{Clotting time of test solution}}{\text{Clotting time of control}}$$

Table 1 records the effect of addition of urea on the clotting of plasma by tissue fibrinogen and calcium.

In spite of the very great reduction in clotting time (from 40 to 3 minutes) produced by the tissue fibrinogen, urea is still able to cause an appre-

TABLE 1

CITRATED HORSE PLASMA	UREA SOLUTIONS	FINAL CONCENTRATION UREA	TISSUE FIBRINOGEN SUSPENSION	1 PER CENT $CaCl_2$	CLOTTING TIME	CLOTTING TIME	
						Plasma control*	T. F. control**
cc.	*cc.*	*grams per 100 cc.*	*cc.*	*cc.*	*minutes*		
1	0.00	0.000	0.00	0.2	40.00	1.000	13.20
1	0.00	0.000	0.05	0.2	3.03	0.076	1.00
1	0.05	0.177	0.05	0.2	2.28	0.57	0.73
1	0.05	0.215	0.05	0.2	2.20	0.55	0.71
1	0.05	0.266	0.05	0.2	2.16	0.054	0.69
1	0.05	0.354	0.05	0.2	2.20	0.055	0.71
1	0.05	0.708	0.05	0.2	2.76	0.69	0.89
1	0.05	1.062	0.05	0.2	3.28	0.082	0.05
1	0.05	1.416	0.05	0.2	3.64	0.091	1.17
1	0.05	1.770	0.05	0.2	4.20	0.105	1.35
1	0.05	2.125	0.05	0.2	4.32	0.103	1.39
1	0.05	4.250	0.05	0.2	9.36	0.234	3.09

* The urea-plasma mixtures were held for five minutes in the water bath at 42°C. before addition of tissue fibrinogen.

** The plasma control contained 0.05 cc. of 0.9 per cent NaCl solution in place of tissue fibrinogen.

ciable acceleration of clotting. This is almost certainly due to the liberation of thrombin precursors and therefore is an acceleration of thrombin clotting, which can proceed side by side with tissue fibrinogen clotting. But the series shows, as it did with thrombin clotting, a minimum clotting time followed by a return toward and beyond normal. We can conclude, therefore, that both acceleration and retardation proceed at the same time; with low urea concentrations the former, and with higher the latter process being the more powerful. Since the relative clotting time with respect to the tissue fibrinogen control becomes greater than 1, we can conclude also that urea concentrations above about 1 gram per 100 cc. interfere with tissue fibrinogen clotting.

The action of urea upon tissue fibrinogen. Tissue fibrinogen, unlike thrombin, can be prepared in a fairly high degree of purity. It is therefore possible to study the action of urea upon this clotting element directly.

In the following experiments, as a source of tissue fibrinogen, we used Fibrinogen-Merrell, a 1.5 per cent suspension of lung tissue fibrinogen in 0.9 per cent NaCl solution. Such a suspension is turbid, difficult to filter and easily precipitates. It was thought that since urea has a great power of peptising proteins it might be used to increase the stability of tissue fibrinogen suspensions. But, as we shall show, although it definitely increases the stability of such suspensions, it also so affects the tissue fibrinogen that part of its clotting activity is lost.

In the first experiment 50 cc. of the 1.5 per cent suspension of tissue fibrinogen in 0.9 per cent NaCl solution (Fibrinogen-Merrell) were measured into a small beaker and the whole weighed, with the addition of a

TABLE 2

	CLOTTING TIME
	seconds
1 cc. plasma + 0.1 cc. 0.9 per cent NaCl solution + 0.2 cc. 1 per cent $CaCl_2$	360
1 cc. plasma + 0.1 cc. original tissue fibrinogen + 0.2 cc. 1 per cent $CaCl_2$ solution	40
1 cc. plasma + 0.1 cc. tap water + 0.2 cc. 1 per cent $CaCl_2$ solution	390
1 cc. plasma + 0.1 cc. tissue fibrinogen after treatment with urea and dialysis + 0.2 cc. 1 per cent $CaCl_2$ solution	65

small glass stirring rod. Crystalline urea was then stirred into the suspension until the maximum of clarity was produced. By weighing it was found that 26.6 grams of urea had been added. Since such a concentration of urea will affect clotting systems it was necessary to remove it by dialysis before carrying out clotting tests. Twenty-five cubic centimeters of the urea-tissue fibrinogen mixture were placed in a Schleicher and Schull parchment dialysing thimble and dialysed for 24 hours against running tap water. The level of the dialysing water was always kept well below the level of fluid in the dialyser so that concentration rather than dilution occurred. At the end of 24 hours the contents of the thimble were as turbid as the original tissue fibrinogen suspension. They were diluted to the original volume (25 cc.) with 0.9 per cent NaCl solution.

The clotting power of the final product, and of controls, was tested as shown in table 2.

Apparently the treatment had destroyed part of the clotting activity of the tissue fibrinogen. Reference to a curve of dilution effects (4) would

indicate that the lengthening of the clotting time from 40 to 65 seconds, as observed here, should mean a loss of over three-fourths of the clotting power of the tissue fibrinogen.

To find the action of lower urea concentrations, 1 cc. samples of the tissue fibrinogen suspension were mixed with 2 cc. of urea solutions of varying strengths and the mixtures dialysed for 24 hours against running tap

TABLE 3

Test for total clotting power

UREA BEFORE DIALYSIS	CITRATES HORSE PLASMA	TISSUE FIBRINOGEN (AFTER UREA AND DIALYSIS)	1 PER CENT $CaCl_2$ SOLUTION	CLOTTING TIMES
grams per 100 cc.	*cc.*	*cc.*	*cc.*	*seconds*
0.00	1	0.1	0.2	45
0.15	1	0.1	0.2	47
10.31	1	0.1	0.2	47
15.46	1	0.1	0.2	53
20.61	1	0.1	0.2	55
25.76	1	0.1	0.2	53
30.91	1	0.1	0.2	53

TABLE 4

Test for free cephalin

UREA CONCENTRATION BEFORE DIALYSIS	CITRATED HORSE PLASMA	OLD SERUM	TISSUE FIBRINOGEN (AFTER UREA AND DIALYSIS)	CLOTTING TIME
grams per 100 cc.	*cc.*	*cc.*	*cc.*	*minutes cc.*
0.00	1	0.5	0.1	20.4
0.15	1	0.5	0.1	20.2
10.31	1	0.5	0.1	17.3
15.46	1	0.5	0.1	19.0
20.61	1	0.5	0.1	22.7
25.76	1	0.5	0.1	20.7
30.91	1	0.5	0.1	17.6
0.00	1	0.5	0.1 cc. fresh tissue fibrinogen	17.3

Note: The mixtures of old serum and tissue fibrinogen samples were held for 5 minutes in the water bath at 42°C. before adding to the plasma.

water. As control, 1 cc. of the tissue fibrinogen was diluted with 2 cc. distilled water and also dialysed. On addition of urea there was in all cases a rapid reduction in turbidity of the suspensions. This reduction increased with increased urea concentration. All of the mixtures with urea were more stable, as measured by the speed of precipitation of solids on standing, than was the control. At the end of dialysis all mixtures were

restored to their original volume of 3 cc. by adding 0.9 per cent NaCl solution.

The dialysed mixtures were tested both for tissue fibrinogen and for free cephalin. Their total clotting power was shown by adding 0.1 cc. of each sample to 1 cc. citrated horse plasma, standing for 5 minutes in the water bath at 42°C. and then treating with 0.2 cc. of 1 per cent $CaCl_2$ solution. Table 3 shows the resulting clotting times. One cubic centimeter of the citrated plasma + 0.1 cc. 0.9 per cent NaCl clotted on recalcification in 360 seconds.

Apparently even the strongest urea solution used in this series had caused less than a 50 per cent decrease in the clotting power of the tissue fibrinogen.

The activity remaining to these urea-treated tissue fibrinogen specimens may be due either to tissue fibrinogen or to free cephalin or to both. Both

TABLE 5

	CLOTTING TIME
	seconds
Test for total clotting power:	
1 cc. plasma + 0.1 cc. 0.1 per cent NaCl + 0.2 cc. 1 per cent $CaCl_2$	215
1 cc. plasma + 0.1 cc. original tissue fibrinogen + 0.2 cc. 1 per cent $CaCl_2$	16
1 cc. plasma + 0.1 cc. tissue fibrogen after saturating with urea and dialysing + 0.2 cc. 1 per cent $CaCl_2$	71
Test for free cephalin:	
1 cc. plasma + (0.5 cc. serum + 0.1 cc. 0.9 per cent NaCl)	62
1 cc. plasma + (0.5 cc. serum + 0.1 cc. original tissue fibrinogen)	57
1 cc. plasma + (0.5 cc. serum + 0.1 tissue fibrinogen after saturating with urea and dialysing)	33

of these substances will accelerate the clotting of citrated plasma on recalcification. To test for the presence of free cephalin we made use of the fact that the thrombin present in fresh serum deteriorates on standing but can be reactivated by addition of cephalin, and after such reactivation will, like the fresh serum, clot citrated plasma without the addition of calcium. Since tissue fibrinogen will not clot without addition of calcium this test serves to distinguish between the two clotting factors, tissue fibrinogen and cephalin.

There was obviously but slightly more cephalin in any of the urea-treated specimens than in the diluted tissue fibrinogen control and no more than in the original tissue fibrinogen. The clotting activity of the urea-treated samples was therefore almost entirely due to tissue fibrinogen. We may infer, then, that urea in the amounts here present did partially inactivate the tissue fibrinogen.

The action of a high concentration of urea was obtained by saturating 3 cc. of tissue fibrinogen suspension with crystalline urea, removing the urea by dialysis, restoring the volume to 3 cc. by addition of 0.9 cc. NaCl solution and testing the product for clotting power and for cephalin in the manner already described.

There was obviously much more free cephalin and very much less active tissue fibrinogen in the tissue fibrinogen solution that had been saturated with urea and dialysed than in the original untreated tissue fibrinogen solution.

As a check on these results we used another test. Mills has shown that tissue-fibrinogen is a globulin-cephalin complex and that if the cephalin be removed by extraction with cold benzene, the residue acts as a fairly powerful anticoagulant. The anticoagulant can be reconverted to active tissue fibrinogen by addition of cephalin. The power to reactivate the anticoagulant is, therefore, a good test for free cephalin. Anticoagulant was prepared by long extraction of air-dried lung tissue. The benzene was decanted off and the residue dried in an air current. The residue was then extracted with 0.9 per cent NaCl solution. The extract when filtered off was diluted with 0.9 per cent NaCl solution as to contain about 0.4 per cent protein. Mixtures were made of 0.2 cc. of anticoagulant solution and 0.1 cc. of the urea-treated, dialysed tissue fibrinogen. A control contained 0.2 cc. of anticoagulant + 0.1 cc. 0.9 per cent NaCl. After leaving the test mixtures to stand for varying lengths of time they, and the control, were added to 1 cc. samples of citrated horse plasma and left in the water bath at 42°C. for 5 minutes. Then each was treated with 0.2 cc. of 1 per cent $CaCl_2$ solution and the clotting time measured in the usual way.

The series gave:

	Clotting time *seconds*
1 cc. plasma + (0.2 cc. anticoagulant + 0.1 cc. 0.9 per cent NaCl solution) + 0.2 cc. $CaCl_2$ solution	1860
1 cc. plasma + (0.2 cc. anticoagulant + 0.1 cc. urea-treated tissue fibrinogen. After standing mixed for 7′ 10″)	68
1 cc. plasma + (0.2 cc. anticoagulant + 0.1 cc. urea treated tissue fibrinogen. After standing mixed for 27′ 48″)	56
1 cc. plasma + 0.2 cc. anticoagulant + 0.1 cc. urea treated tissue fibrinogen. After standing mixed for 86′ 56″)	53
1 cc. plasma + 0.3 cc. 0.9 per cent NaCl solution + 0.2 cc. 1 per cent $CaCl_2$ solution	190

These clotting times indicate a high content of cephalin in the tissue fibrinogen which we had saturated with urea and dialysed. The tissue fibrinogen (urea-treated) which was present here would have brought on clotting in the plasma in about 70 seconds, without the anticoagulant. However, when added to the anticoagulant, it not only destroyed the

great inhibitory activity of the latter but more than doubled the tissue fibrinogen activity of the mixture, as evidenced by the shortening of the clotting time down to 53 seconds.

The action of urea upon heat-coagulated tissue fibrinogen. It is known since the work of Ramsden (6), that urea will dissolve heat-coagulated proteins. Since such coagulation destroys the activity of tissue fibrinogen this substance is ideal for the study of the action of urea as a possible reversal of the process of heat-coagulation.

A known volume of 50 cc. of 1.5 per cent suspension of tissue fibrinogen in 0.9 per cent NaCl solution (Fibrinogen-Merrell) was heated to coagulation and about 1 hour longer in a boiling-water bath. The coagulum was

TABLE 6

CITRATED HORSE PLASMA	OTHER ADDITION	1 PER CENT $CaCl_2$	CLOTTING TIME
cc.		*cc.*	*seconds*
1	0.1 cc. 0.9 per cent NaCl solution	0 2	720
1	0.1 cc. tissue fibrinogen	0 2	50
1	0.1 cc. suspension of heat-coagulated tissue-fibrinogen after solution in urea and dialysis	0 2	120

collected on a filter and then dissolved in saturated urea solution. The solution was collected, then dialysed for 24 hours against running water. A precipitate formed in the dialyser was collected and suspended in 50 cc. of 0.9 per cent NaCl solution. The suspension was opalescent, like suspensions of cephalin. It was tested for tissue fibrinogen and cephalin as already mentioned in this paper.

The clotting activity of this specimen may have been due to tissue fibrinogen or to free cephalin. The usual test for free cephalin gave:

TABLE 7

CITRATED PLASMA	OTHER ADDITION	CLOTTING TIME
cc.		*seconds*
1	(0.1 cc. 0.9 per cent NaCl + 0.5 cc. serum)	180
1	(0.1 cc. 1 per cent cephalin emulsion in 9.9 per cent NaCl + 0.5 cc. serum)	5
1	(0.1 cc. suspension of heat-coagulated tissue-fibrinogen after solution in urea and dialysis + 0.5 cc. serum)	15

The content of cephalin in this specimen was high enough to account for a large part of its clotting activity.

The above experiment was performed on June 10, 1924. The suspen-

sion of the tissue fibrinogen which had been heat-coagulated, dissolved in urea and dialysed was kept in the refrigerator until November 23, 1924, and then tested once more for its clotting power. It was unable to reactivate an old serum and was therefore free from cephalin. Tested for tissue fibrinogen 0.1 cc. of the suspension, added to 1 cc. citrated horse plasma and 0.2 cc. 1 per cent $CaCl_2$ solution, produced a clot in 5 minutes. The control, containing 1 cc. plasma, 0.5 cc. 0.9 per cent NaCl and 0.2 cc. 1 per cent $CaCl_2$, clotted in 8 minutes. Control tissue fibrinogen clotted the plasma in 48 seconds. Apparently tissue fibrinogen was now present, but no free cephalin. The tissue fibrinogen (Mills formula) restored is about 1/2460 of original value.

On November 24, 1924, this suspension was again saturated with solid urea and dialysed against running water for 35 hours. The product was then tested for free cephalin and gave:

Clotting mixture	Clotting time *seconds*
1 cc. citrated plasma + (0.1 cc. 0.9 per cent NaCl + 0.5 cc. serum)	60
1 cc. citrated plasma + (0.1 cc. treated tissue fibrogen + 0.5 cc. serum)	31

There was obviously a large amount of free cephalin in the suspension. As a control on the effect of heat coagulation alone, fresh tissue fibrinogen was coagulated in a boiling water bath, the coagulum filtered off and suspended in 0.9 per cent NaCl solution to bring it to its original volume. Tests for tissue fibrinogen action and for free cephalin in this specimen gave:

	Clotting time *seconds*
Test for tissue fibrinogen:	
1 cc plasma + 0.1 cc. 0.9 NaCl + 0.2 cc. 1 per cent $CaCl_2$	215
1 cc. plasma + 0.1 cc. boiled tissue fibrinogen − 0.2 cc. 1 per cent $CaCl_2$	224
Test for free cephalin:	
1 cc. plasma + (0.1 cc. 0.9 per cent NaCl + 0.5 cc. serum)	60
1 cc. plasma + (0.1 cc. boiled tissue fibrinogen + 0.5 cc. serum)	68

Heat coagulation, therefore, did not liberate free cephalin and did completely destroy the clotting power of the tissue fibrinogen.

This experiment has been repeated with exactly the same results. Urea always liberates cephalin from heat-coagulated tissue fibrinogen and at the same time appears to reverse the action of heat upon the protein, for on standing the free cephalin disappears and there is a reappearance of part of the original clotting power of the tissue fibrinogen.

In conclusion, then, we interpret our findings as indicating that urea may retard tissue fibrinogen clotting in much the same concentrations as it does thrombin clotting. Higher concentrations of urea, especially up to saturation, definitely disintegrate the tissue fibrinogen, splitting off free cephalin in large amounts. This is accomplished even better by boil-

ing the tissue fibrinogen-urea mixture. Whether the urea further disintegrates the protein fraction of tissue fibrinogen we cannot say, but we do know that it does not destroy its cephalin combining capacity.

SUMMARY

1. Saturation of a 1.5 per cent suspension of tissue fibrinogen in 0.9 per cent NaCl with solid urea at room temperature (about 23°C.) liberates free cephalin and destroys the clotting activity of the tissue fibrinogen.

2. Under the same conditions, 53 grams of urea added to 100 cc. of the tissue fibrinogen suspension destroys a considerable part of the tissue fibrinogen.

3. Urea added to tissue fibrinogen suspensions up to a concentration of 31 grams per 100 cc. has less effect. Thirty-one per cent urea destroys only about half the original tissue fibrinogen clotting power.

4. If heat-coatulated tissue fibrinogen be dissolved in saturated urea, then, after removal of urea, free cephalin is found present in high concentration. On standing, the cephalin disappears and a fraction of the original tissue fibrinogen is reproduced. Saturation with urea will again liberate a large quantity of free cephalin. These results indicate that urea, at least partially, reverses the change which heat has produced.

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FURTHER STUDIES ON THE BASAL METABOLISM OF MAYA INDIANS IN YUCATAN

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Among the several races being studied in connection with the comprehensive program of the Carnegie Institution of Washington for investigating racial metabolism are the Mayas in Yucatan. These observations with the Mayas are made in connection with anthropological and archaeological investigations, likewise carried out by the Carnegie Institution of Washington, Harvard University and other organizations. The first study, by Williams and Benedict (1928), brought to light an unusual situation in that it was found for the first time in studying different races that the average metabolism of these Mayas was somewhat above the basal metabolism of North American whites. Other observers of sub-tropical peoples have noted on the average a lower metabolism, at times markedly lower, and in no instance has a general picture been recorded of a metabolism above that for the whites. Recognizing the unusualness of this finding, Williams and Benedict took every precaution in the analysis of their data to rule cut any possible errors. The protocols were most carefully examined, and the calculations were all frequently controlled and checked. Nevertheless, the important conclusion remained that a group of 32 male Mayas had shown a metabolism averaging 5.2 per cent above the Harris-Benedict standards for white men of similar age, weight, and height living in the northern part of the United States. Secondly, there seemed to be a slight tendency, although not sufficiently strong to consider it as a definitely established factor, for those of the more purely Mayan blood to have a somewhat higher metabolism than those of mixed blood. This factor, however, depended so largely upon the differentiation of the several degrees of purity of Mayan blood, that this point was not stressed. A third fact brought out in this first study was that often extremely low pulse rates were found, pulse rates rarely noted in Caucasians. Thus, twelve individuals had a pulse rate below 50, and four had rates of 44 or lower. Such a number of low pulse rates in a small series is extraordinary. Fortunately, it became possible to study further this most interesting

group of natives on the occasion of the "Yucatan Medical Expedition of 1930"[1]

PLAN OF WORK AND TECHNIC. For the basal metabolism measurements our technician, B. L. Bennett, was carefully trained at the Nutrition Laboratory by E. L. Fox in all the niceties of technic of the field respiration apparatus (Benedict, 1927, 1928, 1929), an apparatus especially devised for studies of this character and an apparatus exactly the duplicate of that used in the former expedition. Thus the question of dissimilar technic could be entirely ruled out. All the Mayas tested by us came to the laboratory in the evening, slept there, and were examined early in the morning. Meanwhile, they were not allowed food or drink of any kind.

To test this small superimposed factor of race on the ordinary basal level we would have preferred to have the best physiological apparatus for studying respiratory exchange. The more recently devised helmet (Benedict, 1930) with blower and the absence of valves is, we believe, the most physiologically sound of the various types of apparatus because of the absence of breathing appliances and nose clip. Unfortunately this type of apparatus requires electric current, which is seldom available in the field. On innumerable occasions, however, the field apparatus has been checked by alcohol check tests and by the so-called "physiological check tests," that is, alternate measurements on a well-trained subject on the field apparatus and several of the other well-known types of laboratory respiration apparatus, and we are convinced that the field apparatus gives most trustworthy results. Since all the studies in this comprehensive investigation of racial metabolism are being made with the identical kind of field apparatus, any conceivable systematic error would be common to all studies.

After a period of most satisfactory training, during which Mr. Bennett gave every evidence of being a skilful operator, the entire equipment was shipped to Yucatan and observations were carried out there from January 21, 1930, until March 25, 1930. For the data we have to offer we are indebted to the tact and technical skill of Mr. Bennett, who made all the direct measurements.

In planning this new investigation it was emphasized 1, that evidence of a high metabolism should be noted; 2, that every effort should be made to obtain repeated observations on subjects with high metabolism, if found, so as to have measurements not on one day only but two, three, or even

[1] The study to be described was undertaken as part of the program of the "Yucatan Medical Expedition of 1930," which was organized by the Department of Tropical Medicine of the Harvard School of Public Health and which worked under the auspices of the Carnegie Institution of Washington. The observations upon basal metabolism were all made at the Station of the Institution at Chichen Itzá in Yucatan.

four days; 3, that extraordinarily low pulse rates were to be especially watched for, and 4, that inasmuch as the findings of the earlier expedition suggested that the purer Mayan blood had the *higher* metabolism, one should concentrate, if possible, upon the pure Mayas. In selecting "pure Mayas" it was thought important not to rely solely upon the statement of the subject as to race but to discard from the Mayan category all individuals who had alien surnames, curly hair, or features suggesting racial mixture. This plan was acted upon because it had been learned during the work of the previous year that individuals living in a community in which Mestizos predominate nearly always call themselves Mestizos, that in Mayan communities the reverse is true, and that the pure Maya rarely assumes a Spanish surname.

The technic employed by us was exactly the same for all cases. It consisted of a series of observations to record the age, the weight without clothing, the height, the sitting height, the pulse rate, the respiration rate, the room temperature, and the oxygen consumption per minute. At least two well-agreeing periods for each day were necessary to establish the oxygen consumption, generally three, and rarely was one period accepted. The single period was used only in two cases where we had earlier observations on the same individual, and its purpose was to ascertain whether the metabolism had changed since the earlier measurements made by Williams. Unfortunately, repetitions by us in these two cases were impossible.

We planned to make repeated observations on the Mayan group. This we failed in most cases to do. Out of 26 Mayan subjects we were able on this expedition to secure a second measurement in only 8 cases. A particular effort was made to repeat on individuals who had given us high figures and to re-examine the men of the earlier group studied by Williams. Nine of our subjects had been studied by Williams, but three of these had shown him a perfectly normal metabolism, that is, deviating scarcely at all from the standard for Caucasians. The difficulties of measuring the metabolism of a people like the Mayas, even with a technic reduced to its simplest terms, are greater than would seem possible to the tranquil laboratory worker accustomed to well-trained subjects, or indeed to the laboratory technician employing the routine of a well-ordered hospital. The Mayas, as a rule, dislike to undergo this test. They are not interested in any kind of examination when healthy and, even when they are well paid for their time, more or less moral compulsion is also needed to induce them to serve as subjects. Partly on this account and partly through discharge from employment or absences of individuals for other causes, repetitions which we had confidently expected to make proved to be impossible. We wish to emphasize the fact that our data are fragmentary because, for the reasons above stated, the plans were not fully carried out, and to state that the value of this contribution depends mainly upon its parallelism with the findings of Williams.

At the moment of writing, another expedition is about to start to Yucatan in which there will be still another observer from another laboratory, but likewise trained at the Nutrition Laboratory, who will undertake a third survey of this interesting group of men. It is, therefore, of the greatest value either to confirm fully this unusual finding or to establish by a long series of measurements the true basal metabolism of the Mayas.

DATA. Our data are presented in table 1, side by side with those of Williams for the cases included in both series of tests. The data secured by Williams are indicated by asterisks.

Ages. Difficulties in experimentation with these for the most part uneducated natives are apparent at the outset in the mere matter of recording the ages, for there are several instances where the age record as obtained by Williams clashes curiously with that obtained in our more recent investigation. A typical case in point is that of E. Puc. According to the record of Williams on April 2, 1927, he was twenty-five years old. On January 28, 1930, he said that he was twenty years old, a matter of eight years difference. Since the comparison standards of Harris and Benedict are based upon true and known ages and the age factor is a *real* one, any uncertainty as to age vitiates the final comparison. It was learned in the course of clinical work in 1929 that many of the Mayas are uncertain as to their ages. A young man often states that he is twenty years old, apparently not knowing his real age and seemingly under the impression that if he has passed twenty his exact age is of no moment. Among the Mayan women the age of twenty is considered rather old for an unmarried girl, youth passes early, and the ages of older women are difficult to obtain with any approach to accuracy. Consequently, in certain cases, a closer approximation to the truth can be obtained if the examiner sets down his guess as to age rather than to record an obviously absurd statement of the subject.

Weights. Another difficulty was to secure the weights without clothing. These people are particularly averse to being weighed naked. Usually they wear only a thin pair of pants and a shirt. These were weighed and then the average weight of the clothing was deducted from the weight of the subject with clothes.

Measurements of height and sitting height. The measurement of height is again difficult. We realize that some three years intervened between the two expeditions and the heights in general do not vary any more than would be expected between two observers. Yet we have a gain of 4 cm. in the case of F. Tec, the heights being 158 cm. on April 1, 1927, and 162 cm. on January 28, 1930. To be sure, this man was supposed to be 18 years old at the time of the first measurement and there might have been some growth in the interval. On the other hand, an increase of 4 cm. with C. Mucul is hardly probable, and an increase of 5 cm. with T. Ek (from 158

TABLE 1

Basal metabolism of male Mayas in Yucatan

SUBJECT	AGE	WEIGHT (WITHOUT CLOTHES)	HEIGHT	SITTING HEIGHT	PELIDISI	DATE OF EXPERIMENT	NUMBER OF PERIODS	ROOM TEMPERATURE	PULSE RATE	RESPIRATION RATE	BLOOD PRESSURE		OXYGEN CONSUMED PER MINUTE	DEVIATION FROM HARRIS-BENEDICT PREDICTION
											Systolic	Diastolic		
	years	*kgm.*	*cm.*	*cm.*				*°C.*			*mm.*	*mm.*	*cc.*	*per cent*
U. May	*22	*54.3	*158	*81.4	*101	*Mar. 29, 1927	*3	*23	*54	*15			*203	* −3.1
U. May	*22	*53.4	*158	*81.4	*100	*July 17, 1927	*2	*26	*60	*12			*214	*+3.0
U. May	23	54.4	158	(84.0)	103	Jan. 27, 1930	3	21	53	12	96	74	204	−2.3
U. May	23	55.2	158	87.0	94	Mar. 18, 1930	3	21	56	13			206	−2.1
S. Ceme	*17	*54.8	*155	*81.7	*100	*Apr. 8, 1927	*3	*28	*51	*21			*235	*+10.3
S. Ceme	*17	*52.1	*155	*81.7	*98	*July 13, 1927	*3	*29	*55	*19			*200	*−3.7
S. Ceme	20	57.4	157	86.0	97	Jan. 27, 1930	3	21	55	20	95	60	242	+11.5
E. Puc	*25	*50.2	*150	*79.5	*99	*Apr. 2, 1927	*2	*25	*51	*15			*197	*+2.2
E. Puc	*25	*48.8	*150	*79.5	*98	*July 12, 1927	*3	*27	*50	*13			*209	*+10.0
E. Puc	20	49.0	151	79.0	100	Jan. 28, 1930	3	23	49	9	92	60	207	+5.7
E. Puc	20	51.2	151	79.0	101	Mar. 11, 1930	2	18	50	23			205	+2.3
E. Puc	20	49.9	151	79.0	100	Mar. 20, 1930	2	28	53	20			192	−2.9
F. Tec	*18	*49.8	*158	*79.5	*99	*Apr. 1, 1927	*3	*28	*65	*9			*235	*+15.0
F. Tec	20	50.1	162	83.0	96	Jan. 28, 1930	2	21	56	15	116	66	242	+17.5
G. Chi	*21	*54.3	*154	*80.2	*102	*Mar. 30, 1927	*3	*23	*60	*14			*233	*+12.2
G. Chi	*21	*51.5	*154	*80.2	*100	*July 11, 1927	*2	*24	*51	*11			*224	*+10.8
G. Chi	23	56.7	156	81.5	101	Mar. 12, 1930	3	25	55	12	100	68	218	+3.0
G. Chi	23	55.2	156	81.5	100	Mar. 25, 1930	3	24	55	12			207	−0.9
C. Mucul	*20	*53.4	*148	*80.6	*100	*Apr. 5, 1927	*2	*26	*55	*15			*230	*+13.7
C. Mucul	24	55.8	152	83.5	98	Mar. 12, 1930	3	21	63	16	118	86	224	+8.7
D. Balm	19	53.0	155	81.0	100	Feb. 14, 1930	3	26	62	20	110	90	232	+11.8
G. Cen	22	53.5	163	86.0	94	Feb. 1, 1930	3	17	52	11	102	72	216	+2.2

T. Ek	*22	*53.9	*158	*80.4	*102	*July 11, 1927	*2	*24	*44	*9			*208	*−0.3
T. Ek	*22	*52.4	*158	*80.4	*101	*July 12, 1927	*3	*27	*40	*10			*206	*+0.1
T. Ek	25	56.2	163	83.3	99	Feb. 3, 1930	1	16	40	14	117	70	210	−1.8
S. Mex	21	57.5	166	87.0	96	Feb. 5, 1930	3	23	59	17	110	69	215	−3.4
V. Ukan	30	57.9	163	87.0	96	Feb. 5, 1930	3	24	57	15	118	80	227	+6.8
J. Cene	25	47.2	155	81.5	95	Feb. 6, 1930	2	18	56	14			203	+6.6
J. Ceme	32	51.6	161	86.0	93	Mar. 20, 1930	2	26	60	17			214	+8.9
J. Ceme	32	51.6	161	86.0	93	Mar. 21, 1930	3	27	55	16			204	+3.8
M. Yum	17	45.3	151	82.0	94	Feb. 6, 1930	2	19	56	20	92	68	224	+17.0
S. Hoc	25	55.8	168	87.0	95	Feb. 7, 1930	3	18	54	12	100	70	270	+24.6
D. Mex	15	46.2	155	81.5	94	Feb. 11, 1930	2	22	59	20			203	+2.5
S. Tama	19	52.5	167	85.0	95	Feb. 13, 1930	3	22	58	16			235	+9.2
S. Canul	24	50.2	156	82.5	96	Feb. 11, 1930	2	20	56	14	90	70	218	+10.2
S. Canul	24	50.8	156	82.5	96	Feb. 21, 1930	3	21	51	13			213	+7.0
T. Canul	15	51.2	158	81.0	99	Feb. 14, 1930	2	23	57	15	98	70	226	+7.5
S. Cupul	28	56.2	158	85.0	97	Feb. 16, 1930	3	23	65	22			221	+6.7
Ao. Cupul	19	49.0	155	81.5	96	Feb. 18, 1930	2	22	62	26			246	+23.2
F. Ma	25	56.2	165	82.5	99	Feb. 21, 1930	2	19	56	22	110	78	187	−13.2
F. Na	21	50.8	159	81.4	99	Feb. 21, 1930	2	21	50	28	100	68	201	−1.6
An. Cupul	*20	*51.6	*160	*80.4	*100	*July 16, 1927	*5	*26	*61	*9			*209	*+0.7
An. Cupul	19	55.2	162	83.5	98	Mar. 16, 1930	1	28	60	14			221	+1.9
G. Chan	*21	*48.9	*145	*78.2	*101	*Mar. 28, 1927	*3	*27	*47	*15			*202	*+6.2
G. Chan	*21	*46.2	*145	*78.2	*99	*July 12, 1927	*3	*26	*42	*13			*191	*+3.9
G. Chan	25	46.6	148	80.0	97	Mar. 16, 1930	3	24	53	14			175	−4.9
G. Chan	25	46.6	148	80.0	97	Mar. 17, 1930	3	26	50	15			162	−12.0
H. Toyuc	24	52.5	164	86.5	93	Mar. 21, 1930	3	26	59	17			218	+4.8
H. Toyuc	24	52.8	164	86.5	93	Mar. 24, 1930	2	24	51	21			219	+4.9

[1] Sitting height computed by adding 5 cm. to one-half the total height.

cm. in 1927 to 163 cm. in 1930) at the age of not far from 22 to 25 years seems incredible. It is believed that in every case unusual care was taken in both studies to secure these measurements, although we did not have all the equipment of the anthropometrical laboratory.

The sitting heights were unusual and in many cases they agree remarkably well. On the other hand, there were differences as great as 5 and 6 cm. for the sitting height measurements alone. The sitting height measurement according to Dreyer (1921) requires a technic that is admittedly not extremely simple. The chief value to us of having the sitting height was to compute the pelidisi of Pirquet (1922) as an index of the state of nutrition, for it appeared to be desirable to know whether our subjects were well or poorly nourished. The usual clinical classifications of well nourished, moderately well nourished, poorly or very poorly nourished did not seem to be sufficiently precise, and we believe this laboratory has found with other expeditions that the use of the pelidisi of Pirquet has a great advantage over the ordinary, rather gross clinical indications. As can be seen from the body weights, all of these subjects, as is usual among the Mayas, were small rather than large. For the most part they weighed under 55 kgm. The heights were correspondingly short, and the pelidisi in general are under 100. There are no cases of emaciation. The lowest pelidisi is 93. Judging from the pelidisi alone, we are dealing with a group of people who are not under-nourished. Most of the subjects selected were engaged in hard physical labor, none complained of illness, and all appeared to be healthy.

Pulse rate. From the earlier observations of Williams, who found a relatively large number of individuals with a pulse rate under 50 and a number under 45, our pulse rate studies derive an especial interest. In two of the cases reported in table 1 the pulse was recorded as in the forties. For example, T. Ek. had a rate of 40, which we carefully checked and which confirmed a second experiment of Williams in which he found it at 40. In another case in which Williams reported rates of 47 and 42, respectively, we found 53 and 50. Still another of our men, whose metabolism measurement has not been tabulated because only a *one-period* experiment was recorded, had a pulse rate of 46. Moreover, the pulse rates as a group run low as compared with the standard for white men, and thus confirm in a general way Williams' findings of a low pulse rate.

Respiration rate. The respiration rate depends a great deal upon the attachment of the individual to the respiration apparatus. The experience of the Nutrition Laboratory has been that, not invariably but in general, there is a tendency for the respiration rate to become somewhat slower when any breathing appliance is attached. On the whole, the respiration rates are not far from the normal, but with one of our subjects and with three of Williams' the rates are as low as 9 respirations per minute. This is unusual. Most of the other rates are from 14 to 15 per minute.

Room temperature. In the belief that there might possibly be some correlation between the metabolism and the environmental temperature, we noted the room temperatures, which are recorded in table 1. They do not run extraordinarily high. The highest was 29°C. and the lowest 16°C. They average not far from what would be temperate zone temperatures. Comparison between the room temperature and the metabolism simultaneously measured gives little basis for supposing that there is any close correlation between the room temperature and the metabolism.

INTERPRETATION OF RESULTS. The most important measurement we have to consider is that of the *oxygen consumption*, from which is finally computed the heat production. In considering these oxygen measurements we note, first, the regularity of the oxygen consumption with the same individual at different times. This is shown by a comparison of our records with those of Williams and by comparing our own records from day to day, or from period to period. With the first subject, U. May, in the entire series of four days the average oxygen consumption ranges from 203 cc. to 214 cc., a fairly close agreement, but the next subject has a variability of from 200 cc. noted by Williams to 242 cc. in our periods of observation. The third subject, E. Puc, has an extraordinarily regular oxygen consumption varying only from 192 cc. to 209 cc. For the most part the observations can be said to be pretty reasonably regular, although we find a striking exception with G. Chan. This subject was fairly well nourished and was considered well when studied by us, although he had recently passed through a serious illness. Williams' observations of Chan gave values of 202 cc. and 191 cc. and ours of 175 cc. and 162 cc., respectively. This consideration deals only with the actual oxygen measurements per minute, irrespective of changes in weight, height, or age, which must enter into the calculation of the heat production and the deviation of the heat production as calculated from the standard prediction formula of Harris and Benedict (1919). In these predictions, however, we have again the difficulties already mentioned with regard to the age element, which is questionable in this case. There were discrepancies here also in regard to height and a slight difference in weight, which add other elements of uncertainty.

The percentage deviation from the Harris and Benedict predictions has been calculated for each individual day throughout the entire series. The results are recorded in table 1 in the column entitled "Deviation from Harris-Benedict prediction." When there is general uniformity in the oxygen consumption from day to day by the same individual, we would expect to and do find practically the same figure for the percentage deviation in heat production computed by the method of Harris and Benedict. Thus, with the first subject the deviations range from −3.1 to +3.0 in the series carried out by Williams, and from −2.3 to −2.1 in the second series. With ordinary metabolism measurements it is commonly assumed that

when the records for oxygen consumption per minute for two consecutive periods are within 10 cc. of each other, the degree of accuracy is sufficient. In this particular case, however, this latitude permits a deviation from the predictions of from +3.0 to −2.3 per cent if both Williams' and our figures for the first subject are compared. As one looks down through this column, one finds that a large proportion of the values are plus. This, of itself, is a crude confirmation of the earlier finding of Williams that the average metabolism of his group of individuals was above the Caucasian standards. If the average percentage deviation for each individual is used as the basis for comparison (see table 2), a closer analysis shows that the average deviation for our entire series is +5.8 per cent. This is practically identical with the +5.2 per cent recorded by Williams.

TABLE 2

Average percentage deviation of measured basal metabolism of male Mayas from Harris-Benedict prediction

SUBJECT	DEVIATION	SUBJECT	DEVIATION
	per cent		*per cent*
U. May	−2.2	M. Yum	+17.0
S. Ceme	+11.5	S. Hoc	+24.6
E. Puc	+1.7	D. Mex	+2.5
F. Tec	+17.5	S. Tama	+9.2
G. Chi	+1.1	S. Canul	+8.6
C. Mucul	+8.7	T. Canul	+7.5
D. Balm	+11.8	S. Cupul	+6.7
G. Cen	+2.2	Ao. Cupul	+23.2
T. Ek	−1.8	F. Ma	−13.2
S. Mex	−3.4	F. Na	−1.6
V. Ukan	+6.8	An. Cupul	+1.9
J. Cene	+6.6	G. Chan	−8.5
J. Ceme	+6.4	H. Toyuc	+4.9
Grand average			+5.8

Blood pressure. In connection with a larger series of observations not reported in this article, blood pressure measurements were made on certain of our subjects. These are recorded in the blood pressure column in table 1, for both the systolic and diastolic blood pressure. With this particular group the average systolic blood pressure is slightly over 100 mm. and the average diastolic blood pressure is 75 mm. The more extensive series are to be analyzed in another place.[2] On the whole, however, the blood pressures recorded in our table are a little below the general average for male

[2] Report of the Yucatan Medical Expedition of 1929 and 1930, to be published by the Carnegie Institution of Washington.

Mayas of 15 years of age or over, as shown in the larger series. The relatively few blood pressures obtained by Williams were touched upon in the earlier report, in which it was shown that a group of 9 men, whose systolic blood pressure averaged 119 mm., had a high metabolism, 10.7 per cent above the Harris-Benedict prediction. In another group of 14 men having an average systolic blood pressure of 107 mm., the metabolism was only 1 per cent above the prediction. Williams' blood pressures are not consistently higher than those recorded here, but the average for his 23 subjects is higher, namely, 112 mm.

It is difficult from the figures to discover a correlation between metabolism and either blood pressure or pulse rate. We have already pointed out that the pulse rate averages distinctly low, judging from Caucasian standards, and the blood pressure is definitely low rather than high, but the metabolism appears to be high. On the other hand, comparison of the blood pressure of our different subjects shows that in the eight cases having a systolic blood pressure over 100 mm. the deviation from the predicted metabolism is, in most instances, plus and definitely high, yet in at least two such cases it is slightly minus. Moreover, some cases having low blood pressure show a relatively high metabolism. Thus, in the one day experiment with M. Yum, the systolic blood pressure was 92 mm. when the metabolism deviated from the predicted by + 17 per cent. We believe it dangerous, however, to consider experiments of only *one day* on any one subject.

The psychical element entering into measurements which are so strange to these people must produce some effect. It would tend to increase the metabolism and probably to increase the blood pressure and the pulse rate. Indeed, while taking the large series of observations upon blood pressure it was frequently noted that the systolic pressure might drop 10 mm. or more in the course of repeated readings. In these cases the pulse rate was accelerated also. These subjects, as a rule, were women. With whites, similarly, apprehension or other psychical disturbance is almost invariably reflected in a high pulse rate. But the Mayas that served as subjects for basal metabolism measurements, far from having a high heart rate have a low rate, so that, using this index alone, one might be warranted in supposing that excitement was absent among the male subjects used for basal metabolism tests.

SUMMARY

During a medical expedition to Yucatan in 1930 the basal metabolism was measured of a group of 26 male Mayas at Chichen Itzá. These observations were made to supplement the earlier measurements on Mayas by Williams in 1927, and in 9 instances the same subjects were studied that had been measured during the earlier expedition. The respiration appa-

ratus employed was especially devised for racial studies in the field. These two separate expeditions have both presented data that suggest that the Mayas may have a high basal metabolism associated with low heart rate and low blood pressure. In those cases in which observations were repeated on the same subject there was a general, although not invariable tendency for the metabolism to be slightly lower in the second measurement. This fact suggests that by repeated observations a metabolism figure might be reached that could not be considered abnormally high. This suggestion must not be lost sight of, although our evidence in general is that these people are quiet, phlegmatic, and non-temperamental. Indeed, there was no indication, so far as the pulse rate is concerned, of psychic disturbance. It is particularly fortunate that the Mayas are to be studied again on a third expedition, which will carry out precisely the same metabolism technic and which can take full advantage of the pitfalls uncovered by the study reported here.

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STUDIES IN THE PHYSIOLOGY OF EXERCISE

V. Acid-Base Changes in the Serum of Exercised Dogs

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A number of workers including ourselves have used the dog in studies on the physiological effects of exercise. Recently our work led us to observe the changes in the pH and CO_2 content of the serum of dog's blood in treadmill running. It was not without surprise that we discovered that dog's blood became distinctly alkaline during exercise. Since this is in contrast with changes in human beings, we set about to determine the factors responsible for the difference. The absence of sweat glands in the dog and the consequent overheating with its compensatory panting pointed to overventilation with blowing off of CO_2 as the most likely explanation.

This position was justified by our observations that merely overheating the dog with an electric heater produced changes very similar to those found in the blood of the same animal while exercised on the treadmill. Swimming in cool water which facilitates heat dissipation produced changes in the opposite direction. This we reported in preliminary communication to the XIIIth International Physiological Congress (Steinhaus and Rice, 1929).

To characterize more completely the individual rôles of heat and exercise in the total reaction, we decided to cause the animals to exercise under conditions which would make it possible to vary at will the body temperature without resorting to different forms of exercise. This was achieved by having the animals swim in water at different temperatures. A preliminary report of these experiments has been made (Rice and Steinhaus, 1930).

Our results have been considered from the standpoint of the changes produced in the acid base balance of the blood by 1, heat, and 2, metabolites. The influence of heat on the blood must, in turn, be considered first from its local purely physical effect upon the dissociation of electrolytes, and second from its direct effect via the respiratory reflexes. We have examined the literature with these headings in mind.

Stadie and Martin (1924) and Stadie, Austin and Robinson (1925) from studies in whole blood and serum conclude that an increase in temperature

if unaccompanied by H_2CO_3 loss results in an increased acidity. Bazett (1927) and Bazett and McGlone (1928) report changes in the color of limbs of cats injected with phenol red and submerged in water of widely varying temperature which indicates that also in vivo, locally applied heat lowers pH and cold raises it. Goldschmidt and Light (1925) report increased O_2 and decreased CO_2 in venous blood from the hand held in water below 18°C. When, however, the entire organism becomes warmed and the respiration is augmented these direct temperature effects are overshadowed by the resulting hyperventilation. As early as 1905 Haldane reported increased rate and depth of respiration and lowered CO_2 tensions of alveolar air in men in the very warm Cornish mines and in the incubation room of the Lister Institute. He observed that mild exercise at these temperatures aggravated the dyspnea. Haggard (1920) reports gas changes which indicate to him a more alkaline blood when the body is submerged in water at 43°C. Flinn (1925) found lowered CO_2 capacity and increased pH in blood of dogs exposed to air temperatures of 45° or more. In animals such as the cat and dog where panting offers almost the only route for heat dissipation it is to be expected that the changes due to ventilation will be exaggerated.

The entrance into the blood of acid metabolites also results in loss of blood CO_2 as witness the spurious R.Q.s in excess of 2.0 reported by Hill (1926) for short periods of strenuous exercise. Another most striking picture of this change is found in Henderson's curves (1928, fig. 188, 189) showing simultaneous CO_2 and blood lactate changes in subject C. V. C.

Experimental methods. In all cases blood was drawn, defibrinated and centrifuged under oil, and the serum used for analyses. The tubes containing the blood were kept in a beaker of ice and water except when being directly handled. The pH was determined by the Hastings and Sendroy (1924) bicolorimetric method, using the new bicolorimeter recently developed by Hastings. The colorimeter readings were made at room temperature and corrected to the body temperature existing in the dog when the blood was drawn. These corrections involve the temperature effect 1, on the dissociation constant of phenol red as published by Hastings and Sendroy (1924), and 2, on the pK of serum as reported by Austin and Cullen (1926, p. 49) and may be combined in the single expression:

$$pH_D = 7.78 - 0.0072\ (R - 20°) + \log \frac{15 - X}{X} - 0.013\ (D - R)$$

when pH_D is the pH in the dog at the time of drawing the blood at the body temperature D of the dog at the time. R is the room temperature at which colorimeter readings were made. X is the colorimeter reading (in this case the depth of the acid or yellow form of the dye) and $15 - X$ is

the depth of the alkaline or red form of the dye. The serum CO_2 was determined by means of the Van Slyke and Neill (1924) manometric apparatus. Body temperatures were read from a precision type thermometer inserted into the colon to a distance of at least eight inches. This practice we believe gave us true body temperatures where rectal readings might more easily be affected by the extremes of environmental temperatures employed in the tests.

The routine for all tests was as follows: The animal was kept at rest for at least thirty minutes prior to the drawing of the first sample of blood. A second pre-exercise sample was drawn after five to ten minutes of further resting. The animal was then exercised or overheated. In treadmill exercise, the animal ran at the rate of a mile in twelve minutes up a twenty-two per cent grade on a motor driven treadmill. This arrangement is more taxing on the animal than the somewhat higher horizontal speeds used by many other workers. Rarely can a dog run continuously for 30 minutes on our mill. For overheating a large electric heater was covered with a lattice-work frame, the dog laid upon this and covered with bath towels. In exercising by stationary swimming, the animal was placed in a small swimming-pool where an attendant kept the animal's feet off the edges of the pool, forcing it to swim. In the earlier experiments blood was drawn from the external saphenous vein. Latterly, all samples were drawn from the external jugular vein. The first sample during activity was drawn at the end of six minutes of exercising. This was followed by samples at the end of two more six-minute periods and then samples were drawn at the end of ten-minute periods until complete fatigue occurred. Frequently the last period was prolonged as we waited for indications of exhaustion. Three post-exercise samples were taken, the first at the point of exhaustion, the second after a half-hour of rest, and the third after about two hours of rest. The time required to get a sample of blood varied from one to four minutes. The vasomotor effects of the extreme temperatures occasionally proved troublesome. In order to get a sample while the dog was exercising on the treadmill, the motor was shut off and blood was drawn while the animal stood on the machine. In swimming it was necessary to place the animal on a table beside the swimming pool in order to get the sample. Water temperature and body temperature were taken while the blood was being drawn. Complete fatigue was considered achieved when the animal allowed himself to be dragged on the treadmill, or when his movements in swimming were insufficient to keep his head above water. The dogs used were short-haired male mongrels weighing from 20 to 27 kilograms.

Results. From our study of 29 complete exercise periods on five different animals we have selected the results of seven typical experiments to illustrate our findings. These data are presented in three composite

charts. In figures 1 and 2 mM of bicarbonate per liter of serum is plotted against pH. The CO_2 pressure lines were located in accord with the following equations:

$$\text{pH} = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]}$$

$$[BHCO_3] = CO_2 - H_2CO_3$$

$$[H_2CO_3] = \frac{\text{p}CO_2}{760} \times \frac{X}{22.4} \times 1000$$

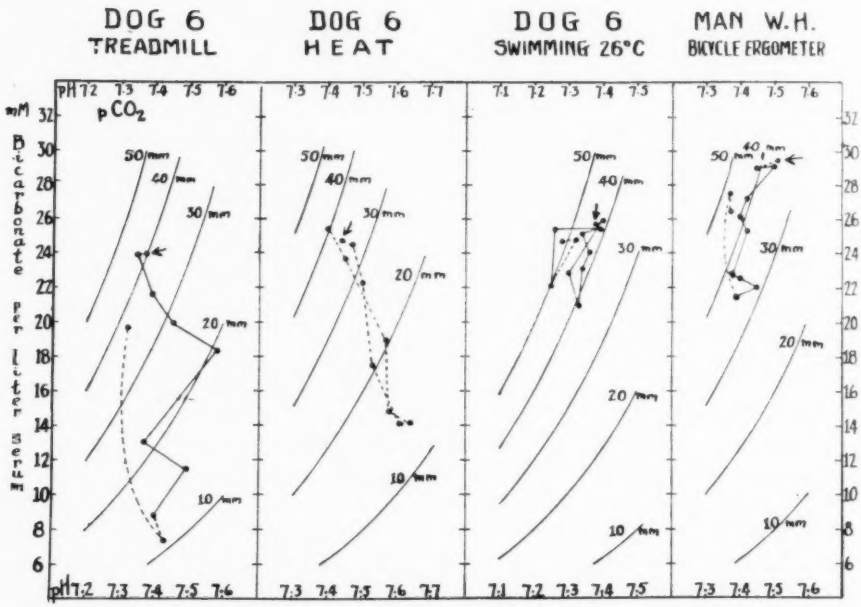

Fig. 1. Acid-base curves of dog and human serum under conditions indicated. Solid lines connect samples drawn during exercise. Broken lines connect samples drawn during resting periods. Arrows point to the first sample drawn in each experiment.

The values $pK' = 6.10$ and $X = 0.510$ were employed. In figure 3 the data of figure 2 are combined and presented on an ordinary time chart to permit more intimate comparison.

Discussion of results. Dog 6 was subjected to treadmill exercise, heat, and swimming at 26°C. (see fig. 1). Dog 19 was caused to swim at water temperatures of 15° 30° and 40° (see figs. 2 and 3). The man

W. H. was observed while riding a bicycle ergometer with mechanical brake (fig. 1).

Heat and treadmill exercise produced comparable changes in the blood of dog 6. There was a rise in body temperature, a rise in pH, and a fall in CO_2 content of the serum. When plotted on an acid base chart as bicarbonate against pH (fig. 1) it is seen that there is a progressive change in the acid base balance in the direction of CO_2 deficit. There is progressive decrease in CO_2 tension to about 12 mm. of mercury. This is the result of overventilation and occurs also in dog 19 swimming at 40° (fig. 2).

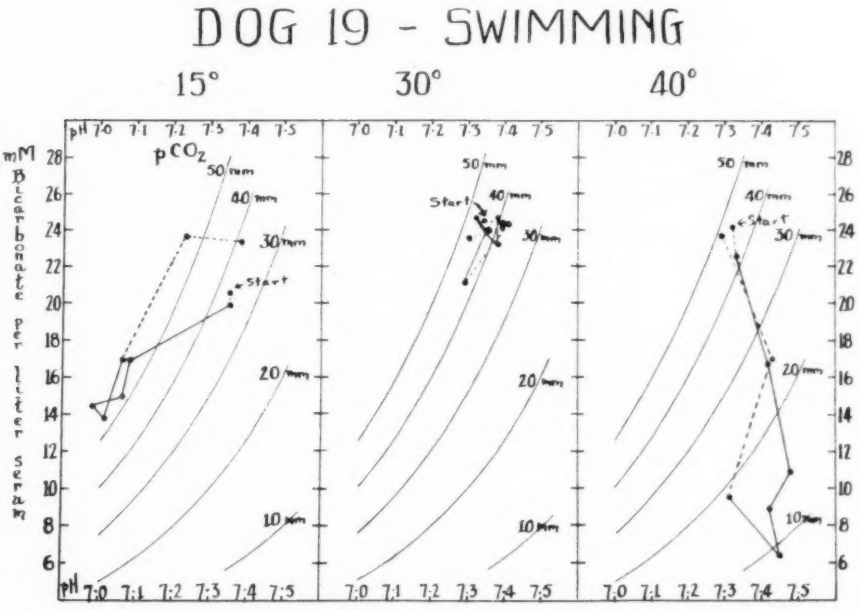

Fig. 2. Acid-base curves of changes in serum of a dog exercised by swimming in water at different temperatures. (For the explanation see legend of fig. 1.)

Overheating can be considered the primary determining factor in each of these cases.

In overheating the hyperventilation which serves to cool the animal causes also a reduction in the CO_2 pressure of the alveolar air and a lowering of the blood CO_2 tension. This leads to an increase in pH, a consequent increase in the base bound by protein and a diminished $BHCO_3$ concentration.

Reductions in the pH and the $BHCO_3$ with stationary or rising CO_2 tensions is the contrasting blood serum picture found in man exercising

on the bicycle ergometer and the dog swimming in lower water temperatures. The dog swimming at 15° shows this trend in its extreme. The entrance of lactic acid into the blood is possibly the determining factor in this set of observations.

Owles (1930) has shown that even in moderately strenuous exercise lactic acid enters the blood. Its reaction with the blood bicarbonates increases the H_2CO_3 and the pCO_2, consequently the pH drops.

The dog swimming at 30°C. shows practically no change in pH, $BHCO_3$ or CO_2 tension until just before exhaustion. Then the pH and $BHCO_3$

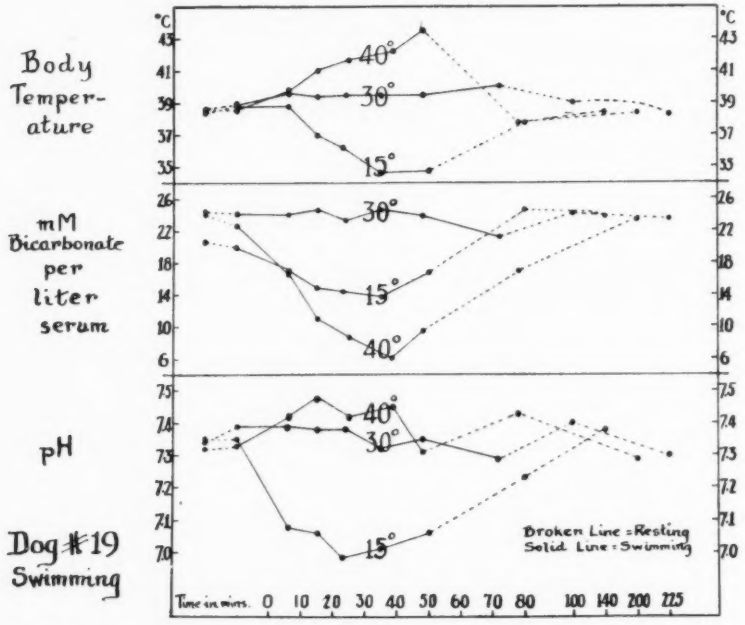

Fig. 3. Time curves drawn to compare changes in body temperature, serum bicarbonate, and serum pH in dogs swimming at various temperatures.

figures drop while the CO_2 tension maintains itself. It is not without interest that at this temperature the dog was able to swim almost half again as long as at the extreme temperatures (seventy-two minutes as compared to fifty minutes,—see fig. 3).

These observations make it quite clear that the changes which predominate in dog's blood during strenuous running on a treadmill are those that result from overventilation, with acid metabolites playing a minor

part. In swimming at lower temperatures, the changes produced by acid metabolites dominate the picture.

Since in man the acid-base changes during ergometer exercise are more nearly like those of the cooled swimming dog than like those of the dog running on the treadmill, it becomes of primary importance that this difference be kept foremost in mind in any attempt to draw conclusions for human physiology from experiments in which dogs are exercised by running. The literature regarding the effects of exercise on gastric secretion is perhaps a case in point. Since 1849 at least twelve different workers have reported conflicting results. The horse, dog, and man have served in experiments in which the exercise varied from pleasant walks to strenuous running and gymnastics. The observations of the most recent workers deserve attention. Delhougne (1926) came to the conclusion that the amount and concentration of gastric acidity was greatly increased during strenuous exercise in man. In changes in the alkali reserve of the blood he finds support for his view that this acid secretion is in the nature of a regulatory mechanism to relieve the blood of acid radicles. In contrast Crandall (1928) and Prikladowisky and Apollonow (1930-a) found that gastric secretion was depressed by exercise in the dog. Crandall reports that his dogs showed temperature rises of as much as 4.6°F. Meyer, Cohen and Carlson (1918) reported diminished volume of gastric secretion with diminished acidity but relatively greater pepsin content in dogs whose body temperature was raised 2–4°F. in the absence of exercise. Though other factors may play a part it seems plausible that the radical difference in the acid-base picture of the exercising man and dog here reported may offer a clew for the unification of such conflicting observations.

In a careful review of the literature bearing on urine changes resulting from fatigue Hastings (1919) found that a similar discrepancy had engaged the attention of two Italians over thirty years ago. In 1887 Aducco showed that the urine of dogs became alkaline in the course of fatigue whereas Benedicenti in 1897 reported that the urine of marching soldiers was always abnormally acid after a prolonged march. In this paper and again in 1921 Hastings reports large reductions in the total CO_2 of dog's blood resulting from exercise. The pH taken in one series showed no marked change. Hastings' dogs ran on a *horizontal* treadmill for much greater distances than did our dogs. He does not report body temperatures but it seems entirely likely that the temperature of his dogs did not rise to the extent that they would have if the running had been done on an *incline* as in our work. With the influence of this factor given its deserved attention our findings may be viewed as logical extensions of trends observed by these earlier workers. Recently Prikladowizky and Appollonow (1930b) have also found marked reductions in blood CO_2 of dogs in strenuous running. These workers report no pH determinations.

SUMMARY

From the analyses of series of blood samples drawn from dogs and man before, during, and after various forms of exercise the following conclusions seem warranted.

1. During strenuous exercise on a 22 per cent grade motor driven treadmill the serum of dog's blood shows a more alkaline pH coincident with reduction in the bicarbonate and CO_2 tension. These changes are related in time and degree to a gradually mounting body temperature. Exhaustion in this form of exercise is characterized by alkalosis and overheating.

2. Dogs exercised by swimming in water at temperatures of 20° and lower show rising CO_2 tensions, more acid pH and lowering of the bicarbonates of the serum. An otherwise steady body temperature drops below normal as the animal nears the exhaustion point.

3. While swimming at water temperatures between 25° and 30°C. a minimum of disturbance is noticed in the dog's acid-base balance and body temperature until just before the correspondingly postponed fatigue point is neared.

4. Overheating without exercise, and swimming in water with temperatures around 40°C. show serum changes in dogs blood similar to those found in treadmill running.

5. The blood changes in man during ergometer exercise though quite opposite to those of the dog exercised by running, parallel closely those of the dog swimming in water at moderate temperatures.

6. Our findings emphasize the need for observing the greatest caution in attempting to translate the results gained from the study of exercised dogs into human physiology unless the differences here described have been given full consideration. In this light, swimming at appropriate water temperatures suggests itself as being preferable to treadmill running when dogs are used in exercise experiments.

We wish to acknowledge the services of Mr. Leslie Page who made the pH determinations here reported for dog 19. We are indebted to Prof. A Baird Hastings for valuable help in the selection of methods and the interpretation of findings.

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A NEW CHART FOR THE INTERPRETATION OF ACID-BASE CHANGES AND ITS APPLICATION TO EXERCISE

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A method of graphing on triaxial coördinate paper, the theory of which will be described in a later paper by one of us, has been found to be very well adapted for the presentation of data secured in the analysis of the acid-base balance of the blood. Since cross-section paper of this nature is ruled with equal spacings between the lines, it is adapted for plotting data related according to the equation $ax + by + cz = K$. It is seen that the Henderson-Hasselbalch equation, $\text{pH} = pK + \log \frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$, is admirably adapted for plotting on this type of paper when it is placed in the form $\text{pH} - \log \text{BHCO}_3 + \log \text{H}_2\text{CO}_3 = pK$ where pH corresponds to ax, $-\log \text{BHCO}_3$ to by, and $\log \text{H}_2\text{CO}_3$ to cz. In place of $\log \text{H}_2\text{CO}_3$, one may substitute log CO_2 tension x F, when F is an appropriate factor including the solubility coefficient of CO_2 in serum. The position of the coördinates may be most readily understood by reference to the figures of a clock. On such a chart constant pH values run in the direction of XII and VI o'clock, constant BHCO_3 values in the direction of IV and X o'clock, and the constant CO_2 tensions in the direction of II and VIII o'clock. In the graphs here presented we have subsequently indicated corresponding numerical values for those factors which are commonly expressed numerically.

A point on such a chart is determined providing any two of the three variables, pH, BHCO_3 or CO_2 tension are known. This spot represents the acid-base picture of the blood at that moment. The charts are drawn with the normal acid-base picture of the blood as the geometric center. The significance of deviations in various directions from this center are indicated in the central figure. The "excess" and "deficit" lines have been empirically determined by experiment. The "constant" lines are such by virtue of their axial position. Thus, for instance, if any series of blood samples shows a deviation from the center on a pathway parallel to the constant pH line, it is obvious that this blood is keeping a constant pH at the expense of changes in the other two factors.

We have found it instructive to plot the data from dog's blood presented

in the preceding paper (Rice and Steinhaus, 1931) on such a chart. From an examination of the six peripheral figures in the accompanying figure the fundamental differences in blood changes under various conditions of exercise in the dog are clearly indicated. Thus in swimming at 15° the

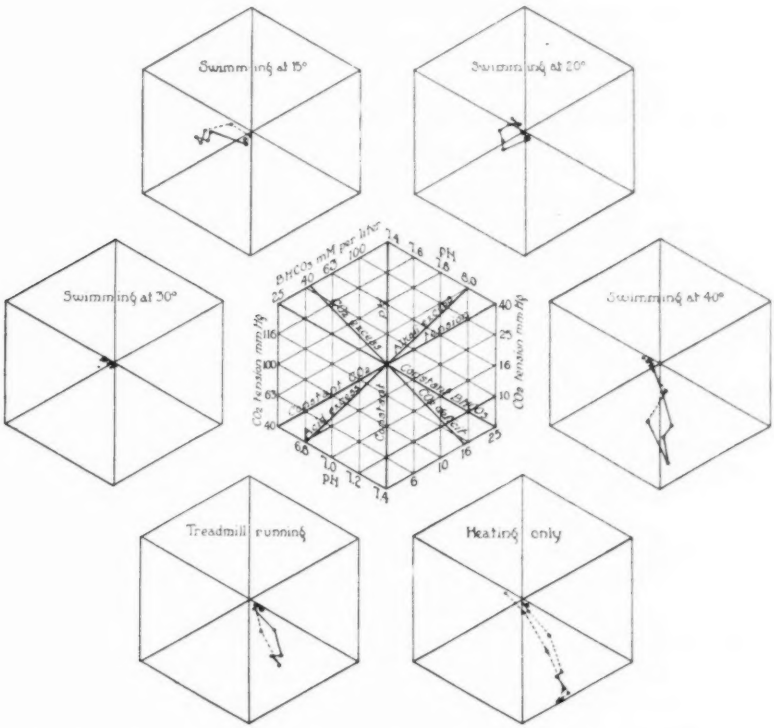

Fig. 1. Acid-base changes in the serum of dogs exercised by swimming in water at indicated temperatures, by running on a motor-driven treadmill (1 mile in 12 minutes on 22 per cent grade), and by overheating without exercise. Samples drawn during rest periods are connected by dotted lines. Those drawn in exercise periods are connected by solid lines. The first sample drawn is in each case indicated by a circle. In the central figure are shown coördinate and other lines which apply for each of the peripheral figures.

analyses of successive samples indicate that the dog's blood is travelling a pathway halfway between acid excess and CO_2 excess. The depression of respiratory movements observed in the dog under these conditions is reflected in this picture. At 20° the disturbance is less and the direction

is that of acid excess with constant CO_2.[1] At 30° the normal equilibria are maintained with only slight modifications. At 40° the path leads along the constant pH axis, its position midway between acid excess and CO_2 deficit indicates the nature of the trends which are balanced to make this possible.

Treadmill running of the strenuous nature described in the preceding paper and heating only are shown to produce changes distinctly in the direction of CO_2 deficit with acid playing a very minor rôle. This is exactly what one would expect to result from the hyperventilation and increased body temperature observed in the animal under these conditions.

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[1] This set of data is not presented in the preceding paper.

OSMOTIC PRESSURE, BOUND WATER AND EDEMA IN PERFUSED HEARTS[1]

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In a paper dealing with the influence of hydrion concentration on the water uptake in perfused rabbit hearts, we (1930) reported general agreement between the behavior of such living and working muscle tissue and similar well recognized effects produced in inanimate hydrophilic colloids by variations of acidity. This effect of the hydrogen ions, it is reasonable to assume, cannot be directly modified to any considerable extent by the permeability of the heart tissues, for both water and hydrogen ions can penetrate into all of them. If, however, the perfusing fluid should contain molecules sufficiently large, or colloidal dispersions of hydrophilic material, then permeability might be a factor. Certainly in the intact animal organism both osmotic pressure and colloidally bound water are assumed to influence the water equilibria in the various tissues.

To test these ideas experimentally we first perfused rabbit hearts exactly as reported in the former paper. The perfusion fluid which we used, however, contained 5 per cent sucrose. In all these experiments the hydrion concentration was constant throughout each perfusion. When the weights of the heart were plotted against time for the different hydrogen-ion concentrations used, we obtained a set of curves practically identical with those for standard Ringer-Locke's solution. That is, in the presence of the sucrose we again found a minimum in the water uptake at pH 8.5. On either side of this point, as at pH 8.2 or 9.1, we found greater water imbibing power, and still farther away, as at pH 7.0 and 9.5, this power again fell off. Moreover, the percentage gain in weight in each case was also about the same as in the absence of the sucrose. Since the osmotic pressure of these solutions was 40 to 50 per cent greater than before, it is evident that under these conditions osmotic pressure cannot be significant in determining the amount of water a perfused heart can absorb. Likewise, sucrose itself cannot have a specific effect.

If the concentration of the sucrose be increased to 10 per cent, the solu-

[1] Read before the Biological Division of the American Chemical Society, Atlanta, Georgia, April 7–11, 1930.

tion becomes viscous and the coronary flow much less; the heart will not beat as long. Nevertheless, a perfused heart can absorb considerable fluid from a solution with an osmotic pressure nearly double that of blood (fig. 1, *a*).

Attention is called to the sharp but temporary loss in weight of this heart which was revealed by a weighing made at the end of the first minute of perfusion. Apparently the permeability of the tissues is small enough to delay the passage of the sucrose solution into the interior of the heart but

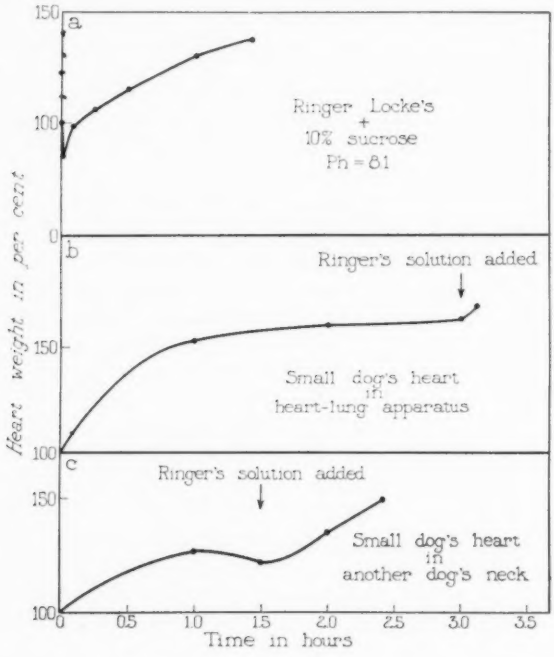

Fig. 1

large enough to permit rapid passage outward of the tissue fluids toward the solution of greater osmotic pressure. This permeability is, therefore, greater than that of a true osmotic membrane. The subsequent gain in weight shows that the perfusing fluid is slowly penetrating into the tissues.

The effect of approximately doubling the osmotic pressure was also observed for a perfusing fluid differing from standard Ringer-Locke's solution only in that its content of sodium chloride was 2 per cent. With this solution of a viscosity not much different from the standard solution the heart beat was vigorous and the coronary flow was good. Such a heart

more than trebled its weight in an hour and a half at pH 8.2. Here again a sharp but temporary loss of weight could be detected if a weighing was made soon after the perfusion was started.

Since the permeability of the heart tissues was obviously too great to permit the osmotic pressure of simple substances in true solution to be more than temporarily effective, suspensions of larger particles were next resorted to. Gum acacia was freed from excess calcium by adding a weighed amount of sodium carbonate to its suspension in hot water. After filtering, the hydrogen-ion concentration was made approximately 7.0 by addition of hydrochloric acid, the final adjustment being made after boiling off the carbon dioxide. Two liters of perfusing fluid were then prepared by adding to the calculated weight of sodium chloride thus formed enough more of this salt, together with distilled water and the usual amounts of the other salts and glucose, to make the whole a Ringer-Locke's solution containing in addition 9 per cent gum acacia. The final pH was 7.4. The time weight curve for this perfusion fluid is similar to that for 10 per cent sucrose which was shown in figure 1, *a*. The coronary flow was much larger, however, and the heart beat for a much longer time. The recovery after the preliminary loss of weight was slower. In two and a half hours the weight had more than doubled. It is evident that either the heart tissue overcame the osmotic pressure and colloidal binding force of the gum or else both gum and water passed into the heart. The latter is probably the case since the initial drop shows the tendency of the solution to absorb water from the heart. In this case, as in the experiments with hypertonic Ringer-Locke's solution containing sucrose or excess of sodium chloride, the perfusion medium extracts fluid from the heart as long as there is even a temporary boundary between the medium and the interior of the heart. Since we know that the salt and sugar soon penetrate into the heart, we assume that the permeability of this boundary is somewhat too large to enable the osmotic pressure of these solutions to continue to extract water from the heart. With the gum dispersed as a sol the force of its osmotic pressure is augmented by the water-binding power of the hydrophilic colloid. However, if a boundary cannot be maintained between this dispersion and the interior of the heart, then after the first outward rush of liquid, the slow inward diffusion of the gum would reverse the loss of weight and duplicate the picture for the solutions made hypertonic by additions of sucrose or excess of sodium chloride. The situation here is of course quite different from that existing at the boundary of an erythrocyte. The interface between such a cell and blood or ordinary solutions is of such a nature that osmotic forces can produce permanent shrinking or swelling of the cells according to whether the medium around them is hypertonic or hypotonic. Then, too, the proportions of the various salts in the interior of such cells is different from that of the blood serum in which they natu-

rally exist. In other words the permeability of the boundary of the erythrocite, whatever its nature, is different from that of the tissues of the perfused hearts.

Similar results were obtained using dispersions of soluble starch and egg white. The exact mechanism by which these colloids bind water is of course not perfectly understood. Some water appears to be adsorbed closely to the surface of the sol micellae and can be separated from them only by a comparatively large expenditure of energy. Other water molecules not actually touching the surfaces of these micellae must still be close enough to them to be within their spheres of influence and hence be more or less strongly attracted by the colloids. There is also to some extent the usual force of osmotic pressure. The combination of these forces is such, then, that as the hearts are perfused, water at first passes out from the heart tissues to dilute the suspensions. If the hearts were impenetrable to these colloids, the equilibrium would be maintained in this condition. This observation seems interesting in view of the fact that a considerable quantity of 6 per cent gum acacia solution is now being used intravenously instead of blood in cases of surgical shock. Since saline or Ringer-Locke's solution is of little permanent value for this purpose, theory of the success of the gum has been as follows: The particles of gum acacia are too large to be rapidly excreted by the kidney or to leak fast out into the tissues, and since much of the water is colloidally bound to the particles of gum acacia it, too, stays in the circulation. Thus the blood volume is kept up and shock is relieved or prevented.

Walker and Keith showed, after injection of solutions of gum acacia that the gum acacia stays in the circulation for days. Furthermore, after an injection of 6 per cent gum acacia they showed that the kidneys can remove a larger amount of the water from the circulation when the tissue reservoirs are well supplied with water than when they are depleted. This indicates that the gum acacia can in turn extract the water from the tissues. Thus the gum acacia exercises some hold on the water and its degree of dispersion prevents it from leaving the circulation.

Therefore it seems that either the heart muscle is more permeable than other tissues in the body or else its permeability becomes greater after it has been removed from the body. Such a change could be due possibly either to denervation or to a different chemical environment, since in many ways Ringer-Locke's solution is not even a close approximation to blood. The importance of the environment is shown by the fact that mere traces of certain substances may have a profound effect, either helpful or harmful, on the vigor of these perfused hearts. However, both these experiments and those reported in the former paper were done on an apparatus with which hearts were being perfused almost daily and with which, if the technic and perfusion solution were kept constant, the results were also constant.

In an attempt to study further the differences between perfused hearts and normal hearts, we did certain experiments on the more or less intact animal. Since operative procedures are simpler on the dog, that animal was chosen for the remainder of the work. For comparative purposes we first perfused a heart from a small dog in the standard way. The time-weight curve can hardly be distinguished from a curve for a rabbit heart under similar conditions. The heart more than doubled its weight in an hour. Apparently the hearts of rabbits and dogs are essentially alike in these respects.

Always, in order to measure edema, we have weighed the hearts at the start of the experiments and then at intervals during the experiments. But a heart cannot be removed and weighed in an experiment that is to be performed in the intact animal. Moreover, there seems to be no definite relationship between the weight of a dog and the weight of its heart. If normal hearts had a fairly constant content of water, then a determination of the dry material in a weighed amount of heart muscle, taken at the end of an experiment designed to increase the content of water of the heart, would enable us to calculate the normal weight of that bit of tissue and so measure the edema. However, experiments in which such muscle was finely minced and dried for two days at 110°C. showed that the water content of supposedly normal dogs' hearts varied more than the amount of edema we might expect to produce in the more or less intact animal.

Although we performed some experiments in denervating the hearts, in some of which the osmotic pressure and colloidal content of the blood were drastically changed, we could not prove the presence of edema. For although the heart would swell and tighten the pericardium, or if that were cut, would continue to enlarge much farther, we could not distinguish decisively between dilatation and edema.

We tried next an experiment in which a small dog's heart was perfused with the blood of a heart-lung preparation; that is, the aorta of the small heart was connected to the circuit and the blood flowing out by way of its coronary vessels was returned to the main circulation from time to time. The small heart could be removed at intervals and weighed. The large dog's blood in the heart-lung preparation was heparinized to prevent clotting and additional completely defibrinated dog's blood was added. The time-weight curve is shown in figure 1*b*. The small heart absorbed about 50 per cent of its weight of fluid but did not gain nearly as much weight as a similar heart perfused with Ringer-Locke's solution such as we have already described. Then 400 cc. of Ringer's solution were added to the circulation and in about seven minutes the large dog's heart failed. The small heart which was still beating vigorously was quickly removed and weighed. In the seven minutes of contact with the increased amount of free water it had made a distinct gain in weight.

As a final approach to a denervated heart in the otherwise intact organism, the following experiment was done. A large dog was etherized, its carotid artery and jugular vein were exposed, and its blood was partially defibrinated and heparinized. The heart of a small dog was then removed under ether anesthesia and its aorta was connected by cannula to the larger dog's carotid. This heart was supported so that the blood from its pulmonary artery fell into a funnel from which it slowly entered by cannula into the larger dog's jugular vein. The perfused heart could thus be removed and weighed. It beat vigorously until the failure of the circulation stopped its blood supply. Figure 1 *c* shows the curve for the perfused heart. Again we observed a small amount of gain in weight with the heart on dog's blood. Then Ringer's solution was injected intravenously at a rate so that 1 liter went in during the hour that the experiment lasted thereafter. Absorption of water was noticed immediately.

Perfused hearts, then, under all conditions studied gained weight. The nearer we could approach to the normal and measure the edema or increase in weight, the less it was. This uptake of water may possibly be due partly to denervation but it is markedly affected by certain alterations in the composition of the fluid in contact with the heart. Increased permeability in the isolated hearts probably prevents the factors of osmotic pressure and colloidally bound water from exerting the same influence that they would have in the intact animal. At least it is certain that when perfused by the usual technic, doubling the osmotic pressure or introducing hydrophilic colloids into the perfusion solution, does not prevent the hearts from absorbing about the same amount of water that they would absorb from a standard Ringer-Locke's solution at the same hydrogen-ion concentration.

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THE EFFECT OF DIFFERENT PER CENTS OF PROTEIN IN THE DIET

I. GROWTH[1]

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If one examines the various dietaries recommended by different physiologists and dietitians he is impressed by the wide range in the energy value and the per cent of protein in the diet. The energy value of these dietaries varies from as low as 2324, to as high as 3436 calories. Experiments are on record in which the range between the low and high has been far greater. The per cent of protein ranges from 9.75 as recommended by Chittenden (1904) to as high as 18 per cent as given by Ranke (1900, 1916). Many of these diets were derived from data compiled from the average food consumption of well nourished classes of people. Others were based on the results of feeding experiments in which the nitrogen balance was the principal determining factor. The majority of these experiments have been pursued for only a relatively short part of the subject's life and have been concerned mainly with the maintenance of weight, nitrogen equilibrium and ability to perform the daily physical or mental duties during the period of experimentation. So far as we know there has been no organized attempt made to determine the effect of different per cents of protein in the diet on longevity, maximum total efficiency, reproduction, and growth and development of the offspring.

The purpose of this experiment was to determine the effect of continuous feeding of different per cents of protein in an otherwise well balanced diet on the growth, activity, food consumption, reproduction, reproductive span, the number, growth and mortality of the young and the total life span of the albino rat through several generations. We felt that by pursuing this undertaking to a successful end we would be able to determine a per cent of protein in the diet which was best adapted to bring about maximum results in the physiological functions of the rat. In doing this we hoped to establish certain physiological principles which

[1] This research has been assisted by the Department of Physiology, the National Research Council through the Committee for Research in Problems of Sex, the National Live Stock and Meat Board, the Fleischmann Company and their successors, the Standard Brands, Inc., and the coöperation of Albers Bros. Milling Company.

might be utilized in connection with experiments on other animals including man.

The rat is well adapted to this sort of experiment. It has a relatively short life span, it is omnivorous in its dietary habits, becomes sexually mature at an early age, its reproductive activities are rapid and the diet given can be absolutely controlled. The size of the animal makes it possible to house a large number in a relatively small space, and to provide the necessary food at a low cost.

The experiment was carried on in a large attic room containing approximately 160 sq. m. of floor space. It was well lighted by skylights of ribbed glass which diffused the light. The area of these skylights was approximately 25 sq. m. A ventilating fan, which blew the air outside through a roof ventilator, caused a good change of air without drafts in any of the lower portions of the room. A recording thermometer registered the temperature at all times. This showed an annual fluctuation of 12°C. ranging from a minimum of 14° in the winter to a maximum of 26° in the summer. The daily range was seldom greater than 5°, being coldest at night when the animals were normally active and warmest during the day when they were asleep. A recording hygrometer showed a range of moisture from 40 to 70 per cent saturation, with an average of approximately 50 per cent for the winter months and 60 per cent for the summer months. The daily range was seldom more than 20 per cent, being higher during the night. The changes in the environment which occurred would presumably affect each of the groups in a similar manner. Since all the animals were kept in the same room in the same environmental conditions any differences in the results of these groups could be attributed to the one variable factor, the diet.

Five different per cents of protein were used. The lowest was 10 per cent and the highest was 26 per cent. This range is well below and above the average protein intake of man. The intervening diets were approximately 4 per cent apart.

The experiment was started with 90 pairs of rats, 18 pairs being used in each of the five groups representing the five per cents of protein. From the lowest to the highest per cent of protein these groups were designated Protein I, II, III, IV, and V. Each group received its special diet throughout life. The offspring and succeeding generations were continued on the same diet as that of their ancestors.

In order to determine the daily spontaneous activity and food consumption 10 females from each group were segregated in individual revolving cages which were their homes during their life spans. They were removed from these cages only for weighing and mating. This usually required but a few minutes. Matings were made with the male of the original pair. These males and the other pairs were kept in stationary cages. Each of

these pairs was isolated in separate cages. Eight pairs in each group were thus constantly together except when young were born, at which time the male was removed for two or three weeks during the nursing period. Owing to the lack of revolving cages the original males could not be tested for spontaneous activity. Bachelor males and virgin females of the offspring are now being tested for spontaneous activity.

The experiment was started in the spring of 1927 when the animals were approximately at the weaning age, and has been in continuous progress since. The average age in days for each group was: Protein I, 44; II, 29; III, 25; IV, 23; V, 24. All these animals had been reared on a synthetic diet which we had found produced excellent growth, reproduction and longevity. If this food had any special advantage group I was more favored than any of the other groups since these animals were somewhat older at the beginning of the experiment.

The paired animals were weighed monthly. The offspring were weighed daily until weaned, then every three or four days until the age of approximately 50 days. After this monthly weighings were made. Owing to the lack of sufficient cages for housing the constantly increasing number of young we were seldom able to continue the weighing of offspring much beyond 200 days of age when they were discarded to make room for new young. The curve of growth of the offspring, therefore, from this age on to normal death was made from monthly weights of paired offspring for succeeding generations. Approximately 800 rats could be accommodated in our cages. Soon after the beginning of the experiment this number was under continuous observation.

The pairs of all later generation matings were kept in separate stationary cages and were permitted to rear as many litters as possible. All such matings were made from individuals of the same generation. The offspring of original pairs were designated generation 1 and succeeding generations in numerical order.

Diet. An accurately prepared synthetic diet was used throughout the experiment. A careful study was made in the selection of the ingredients in order that all the known amino acids and vitamins were present. After a short time it was found that the diet was deficient in vitamin B. This was especially noted in the offspring of the animals on low protein, group I, and was manifested by greatly retarded growth of the young and often by malformations. Such conditions were not observed in the other groups. The most common deformities consisted of blindness or of a loss of from one-fourth to three-fourths of the tail. This latter process followed a definite order. A constriction would appear about one-fourth the length from the tip of the tail, the distal fourth would become very edematous, often increasing in diameter to four or five times the normal, and finally drop off. If the process continued a second and finally a third quarter

would be similarly disposed of, leaving bob-tails with but a fourth of the tail remaining. Those which were blind remained so throughout life. The lids remained closed and their sunken condition indicated a lack of development of the eye ball. The cause of the trouble was at least partly due to the lack of mammary secretion in the mothers. We at once increased the amount of vitamin B by adding Fleischmann's yeast especially prepared for stock and poultry. After this there were no more malformations and the mothers were better able to nurse the young.

The diet containing the lowest per cent of protein, designated Protein I, consisted of the following ingredients in grams: cornstarch, 5000; whole ground wheat, 2000; whole ground yellow corn, 1000; commercial skimmed milk powder, 400; ground alfalfa leaves, 400; commercial casein, 200; meat scrap, 100; wheat germ, 300; unsalted butter, 500; yeast, 200; sodium chloride, 100; calcium carbonate, 150. These were thoroughly mixed and finally run through a grinder. The distribution of food energy in this diet in per cents was: proteins, 10.3; fats, 12.5; and carbohydrates, 77.2. Approximately 57 per cent of the protein came from plant and 43 per cent from animal sources.

Protein II diet was made by adding 900 grams of meat scrap to 10350 grams of the Protein I diet. This diet contained 14.2 per cent of protein, 14.2 per cent of fats and 71.6 per cent of carbohydrates. Thirty-eight per cent of the protein was from plant sources and 62 per cent from animal.

Protein III diet consisted of 2000 grams of meat scrap added to 10350 grams of the Protein I diet. This consisted of 18.2 per cent of protein, 15.9 per cent of fats and 65.9 per cent of carbohydrates. Approximately 25 per cent of the protein was derived from plant and 75 per cent from animal sources.

Protein IV diet was made by adding to 10350 grams of the Protein I diet 3350 grams of meat scrap. This had 22.2 per cent of protein, 17.7 per cent of fats, and 60.1 per cent of carbohydrates. About 19 per cent of the protein came from plant and 81 per cent from animal sources.

Protein V diet was composed of 10350 grams of the Protein I diet and 5100 grams of meat scrap. This diet consisted of 26.3 per cent of proteins, 19.7 per cent of fats, and 54 per cent of carbohydrates. Fourteen and five-tenths per cent of the protein came from plant and 85.5 per cent from animal sources.

A summary of the diet for each group is given in table 1. It is readily seen that the principal variable factor in these different diets was the amount of meat used. It is also noted that the per cent of fat in the diet increased with the increase in the per cent of protein. The greatest variations occurred in the protein and carbohydrate constituents.

Each of these five diets had an energy value of 3.82 calories per gram. By determining the amount of food eaten we were able to calculate the

energy intake per kilo of body weight in the rat. This was found to average approximately 340 calories. This is about ten times the energy intake per kilo of body weight found in man. However, if a comparison is made of the energy intake in relation to one square meter of surface area in the rat and man the latter shows a greater expenditure of energy. The B.M.R. for man per square meter of body surface is approximately 1000 calories per day. The results of Mitchell and Carman (1926) indicate that the B.M.R. per square meter of surface area in the rat, between the ages of 90 and 190 days was approximately 600 calories per day. Older rats, like in man, show a lowering of the B.M.R.

TABLE 1

Showing the per cents of protein, fats and carbohydrates in each of the diets

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
Protein	10.3	14.2	18.2	23.2	26.3
Fats	12.2	14.2	15.9	17.8	19.7
Carbohydrates	77.5	71.6	65.9	60.0	54.0

TABLE 2

Average ages and weights of the 18 pairs in each group at beginning of experiment

GROUP	MALES					FEMALES				
	Number	Age		Weight		Number	Age		Weight	
		Average	Range	Average	P.E.		Average	Range	Average	P.E.
I	18	51	35–64	108	±0.66	18	38	34–46	91	±0.55
II	18	31	20–40	65	±0.82	18	27	20–39	48	±0.75
III	18	25	21–31	43	±0.49	18	26	21–34	40	±0.47
IV	18	23	20–27	37	±0.18	18	23	21–27	35	±0.30
V	18	24	22–28	39	±0.24	18	24	23–27	33	±0.11

Each of the groups was fed its respective diet daily at a given hour. In order to determine the daily food consumption an amount of food greater than the animal would consume before the next feeding time was carefully weighed and the uneaten portion again weighed at the following feeding time. This was done only for the isolated animals in revolving cages. Those in stationary cages were in pairs and the individual food intake could not be determined.

Owing to the great amount of data that has accumulated the results of this experiment will have to be published in a series of papers under the following captions: Growth, spontaneous activity, food consumption, reproduction and the length of sexual life, the offspring, and the life span and

cause of death. This paper will deal with the growth of the original pairs and such replacements as were made on account of early death of original animals. Due to the incompleteness of the data of the offspring the results of succeeding generations will have to be given in later papers.

Growth. Table 2 gives the average ages and weights at the beginning of the experiment. These weights are normal for animals reared on the diet which had been previously fed. It is noted that the animals in group I were older and had a greater weight than any of the other groups. This may have been a distinct advantage to this group over the others. The

TABLE 3

Average ages at maximum weight and average maximum weights of beginning pairs together with remates on account of death

GROUP	MALES					FEMALES				
	Number	Age		Weight		Number	Age		Weight	
		Average	Range	Average	P.E.		Average	Range	Average	P.E.
I	25	473	241–790	298	±1.77	24	455	154–768	256	±1.35
II	23	510	268–933	326	±1.34	21	560	251–806	280	±1.28
III	22	511	245–793	303	±1.72	21	587	280–921	275	±1.78
IV	23	500	149–882	286	±1.77	23	564	154–886	241	±1.44
V	27	495	259–746	279	±1.44	26	564	183–934	224	±1.44

TABLE 4

Number and per cent of original eighteen animals which died before 500 days

GROUP	MALES		FEMALES	
	Number	Per cent	Number	Per cent
I	4	22.2	5	27.7
II	2	11.1	2	11.1
III	3	16.7	1	5.5
IV	4	22.2	1	5.5
V	7	38.9	7	38.9

results of later generations will throw some light on this possibility. It is also noted that the males were heavier than the females in each group. The weights of both sexes were normal for the ages given. None of these animals had reached sexual maturity.

Maximal weight. Table 3 gives the average maximal weight and age at which it was attained for each sex in each group. This includes not only the 18 original pairs in each group but also such replacements as were made on account of premature death of one or both of the original animals. These replacements enabled us to determine the age at which the animals

ceased to breed. It will be noticed that the range in age at which the maximal weight was attained was large. This was partly due to some deaths occurring at a relatively early age. Some of these would doubtless have reached a heavier weight had they lived a normal life span. In table 4 are given the number and per cent of the original eighteen of each sex which died before 500 days of age. This shows that up to 500 days the males of groups II and III and the females of groups II, III and IV had the least mortality and that group V in both sexes had the greatest.

By consulting table 3 it is seen that both sexes of group II had the greatest maximal weight of any of the groups and that group V had the least average maximal weight. The order of maximal weights from highest to lowest was the same for each sex, the order being group II, III, I, IV and V. This indicates that a diet slightly richer in protein than that given group II (14.2 per cent), would be the optimum for maximal growth.

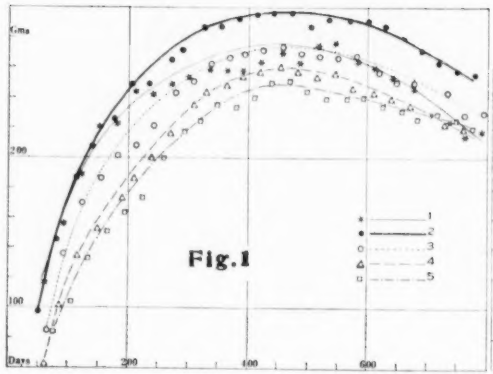
TABLE 5

Average ages and weights at death of beginning pairs together with remates on account of death

GROUP	MALES					FEMALES				
	Number	Age		Weight		Number	Age		Weight	
		Average	Range	Average	P.E.		Average	Range	Average	P.E.
I	25	687	341–1086	194	±1.83	24	764	274–1250	159	±1.08
II	23	766	358–1128	222	±1.44	21	848	469–1108	185	±1.20
III	22	702	330–1172	190	±1.31	21	810	489–1061	198	±2.19
IV	23	671	274–1057	205	±1.40	23	767	222–1276	167	±2.23
V	27	628	344–1182	216	±1.66	26	737	235–1282	161	±1.26

This would correspond very closely to the diet recommended by Voit (1881) for a man weighing 70 to 75 kilos. This diet contained 118 grams protein (15.84 per cent), 56 grams of fat (17.5 per cent) and 500 grams of carbohydrates (67.11 per cent) with an energy value of 3053 calories, or 42.42 calories per kilo of body weight.

Weight at death. In table 5 are given the average weight and age of each sex in each group at death. This shows that the males of group II were the heaviest at death of any of the groups. They also had the longest life span. Group V was the next heaviest, but the length of life was the least of any, being 138 days shorter than that of group II. This weight of 216 grams in group V was partly due to this group having died at an age nearer that of maximal weight thus allowing a shorter period of senile decline. The females of group III had the greatest average death weight and were followed closely by group II. The latter group, however, had the longest average life span. Four of the females of group III had very large



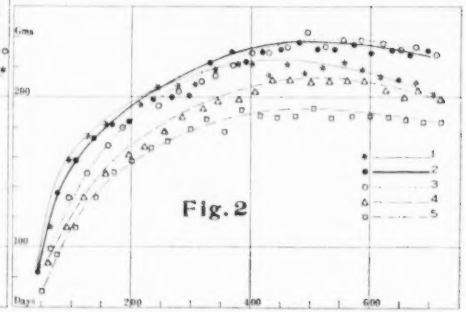


Fig. 1. Curves of average growth of the males of each group. Light continuous line and stars, group I. Heavy continuous line and dots, group II. Dotted line and circles, group III. Dash line and triangles, group IV. Dash-dot line and squares, group V.

Fig. 2. Curve of average growth of the females of each group. Light continuous line and stars, group I. Heavy continuous line and dots, group II. Dotted line and circles, group III. Dash line and triangles, group IV. Dash-dot line and squares, group V.

tumors. The excised tumors often weighed nearly one-half the total weight. One of these rats with tumor intact weighed 380 grams at death. The average death weight of the remaining 17 females of this group was 180 grams. If these abnormal growths were eliminated group III would take second place and group II the first. It is also interesting to note that the life span of the females in each group greatly surpassed that of the males. The average life span for all groups of males was 691 days and the females 785 days. This is in accordance with our previous investigations.

These results show that a diet containing slightly in excess of 14 per cent protein enables the rat not only to reach a greater maximal weight, but also to maintain a heavier weight over a longer life span than any of the other per cents of protein used in this experiment.

Curve of growth. Figure 1 shows the average growth curve of the males in each group. These curves were based on the average monthly weights.

TABLE 6

Average body lengths taken at autopsies

GROUP	MALES			FEMALES		
	Number	Average	P.E.	Number	Average	P.E.
I	20	180	±0.48	21	174	±0.44
II	21	191	±0.44	20	175	±0.27
III	16	178	±0.70	17	168	±0.60
IV	14	178	±0.77	17	174	±0.45
V	14	178	±0.84	20	166	±0.39

Owing to the wide range in the age at which different individuals reached the maximal weight the maximum of the curve of growth—based on weights at definite ages—will not correspond to table 3. Even though the animals of group I were older and heavier than any of the other groups at the beginning of the experiment (table 2) they were soon surpassed by group II which maintained the greatest weight of any of the groups. It is seen that each group reached its maximal growth at approximately 460 days after which there was a gradual decline. Groups I, II and III show practically a normal growth while groups IV and V present a retarded growth which lacked recuperation. These curves of growth of the males again indicate that 14 per cent of protein in the diet approaches very closely to the optimum.

Figure 2 represents the average growth curves of the females of each group. This shows that the age at which maximal growth was reached varied from 425 days in group I to 570 days in group III. It is also seen that groups II and III reached the greatest weight. It is further no-

ticed that groups I and II grew at practically the same rate until about 200 days after which group I showed a retarded growth. The same relation of groups IV and V, as seen in the males, is found in the females. The character and relationship of these curves was no doubt modified by breeding. All these females were mated as often as possible. We have previously shown (Slonaker, 1927) that the bearing of young increases the weight of the mother. Since 50 per cent of the females in group V were sterile their curve of growth would more closely correspond to that of virgins than groups I and II which were 100 per cent fertile, or groups III and IV which had a relatively high per cent of fertility. Experiments are now in progress to test the effect of these diets on virgins and strict bachelors.

In order to determine whether the differences in weight were due merely to changes in obesity or were accompanied by modifications in stature measurements of body length were made at autopsies. The averages are given in table 6. This shows that both sexes of group II surpassed in body length that of any other group. The difference in the males is much greater than in the females. This indicates that a diet containing approximately 14 per cent protein is best adapted to skeletal growth.

SUMMARY

These results indicate that when best growth is considered the amount of protein in the diet should be slightly in excess of 14 per cent. A deviation of 4 per cent less or greater produced no serious results. They further show that as the per cents of protein increase beyond the optimum there was a progressive retardation in growth. Indications are that similar results would obtain if the per cent of protein were reduced progressively below the optimum.

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EFFECT OF DIFFERENT PER CENTS OF PROTEIN IN THE DIET

II. Spontaneous Activity[1]

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In our first paper on this subject, which dealt with the effect on growth (Slonaker, 1931) we gave in detail a description of the plan, purpose, materials and procedure of this research. Only a brief summary will be given in this paper.

The purpose of this research was to determine the effect of continuous feeding of definite per cents of protein in the diet on growth, spontaneous activity, food consumption, reproduction and the length of sexual life, the offspring, and the total life span and cause of death through several generations of albino rats. By carrying this research through to a successful end we hoped to establish results which might be useful in feeding experiments with other animals including man.

Five different groups of animals were used. Each group was fed continuously throughout life and succeeding generations on a specially prepared synthetic diet composed of the same ingredients and containing a constant per cent of protein. Each diet contained all the known amino acids and vitamins.

The groups were designated I, II, III, IV, and V and were fed on diets containing 10, 14, 18, 22, and 26 per cent protein respectively. The extremes represent diets well below and above the average protein intake of man. At the beginning of the experiment each group consisted of 18 pairs of sexually immature rats. The offspring and succeeding generations soon increased this number to several hundred in each group.

This paper will deal with the effect of the different per cents of protein in the diet on the spontaneous activity. Owing to the number of revolving cages available only a limited number of animals from each group could be tested. Ten females in each group taken from the original matings

[1] This research has been assisted by the Department of Physiology, the National Research Council through its Committee for Research in Problems of Sex, the National Live Stock and Meat Board, the Fleischmann Company and their successors, Standard Brands, Inc., and the coöperation of Albers Bros. Milling Company.

were placed in individual revolving cages at the beginning of the experiment. These were their homes during their life spans. They were removed only for weighings and matings which required but a short time. The lack of cages prevented getting the spontaneous activity of representative groups of males at the same time. Such tests, however, are now in progress.

We have used spontaneous running activity as a measure of efficiency. It is well known that an animal which is not getting the food it needs or is ailing in any way is not as active as a normal, well-fed animal. The amount of spontaneous activity, therefore, will depend on the condition of the animal. Since our apparatus is adapted to record automatically only the running activity we have used this activity as a basis for comparison of the value of the different foods used in this experiment as to the effect on efficiency. In doing this we have assumed that the other activi-

TABLE 1

Average age and weight at beginning of experiment of the 10 females in each group

GROUP	NUMBER	AGE		WEIGHT	
		Average	Range	Average	P.E.
I	10	42	34–47	102	±0.658
II	10	21	20–22	30	±0.197
III	10	22	21–23	30	±0.372
IV	10	22	21–38	29	±0.674
V	10	26	24–29	31	±0.060

ties manifested by the rat are in proportion to the running activity exhibited.

In table 1 we have given the average ages and weights of each group when placed in the revolving cages. It will be noticed that group I had the greatest average age and weight and that the remaining groups were nearly alike in age and weight. All these animals had been reared on the same synthetic diet. The animals in group I had been weaned 20 days while those in the other groups were placed in their respective cages at the time of weaning. Group I therefore had the advantage over the other groups in that they had already made the adjustments involved at weaning.

This advantage no doubt accounts in part, at least, for the earlier start of spontaneous activity of group I as shown in table 2. The greater age may also have had an influence in causing the earlier start. Four of the animals in this group averaged approximately 4000 revolutions the first day, three ran 3000 on the third day and one 5000 on the fifth day. The remaining two did not begin until 32 and 60 days after being placed in the cages. There was only one other animal which began running the

first day and this was in group V. The next earliest to start in this group was 30 days.

By consulting table 2 it is seen that there was an increase in the number of days before running activity started from group I to group V. Almost the same relationship occurred in the number of days which elapsed before the activity was in full swing. The average ages at which activity started were, from groups I to V, 53, 73, 73, 92, and 154 days respectively. Whether

TABLE 2

Showing the average days, after placing in cages, of the beginning of spontaneous activity and the average days and number of daily revolutions when the activity was in full swing

GROUP	DAY ACTIVITY STARTED			REVOLUTIONS		
	Age	Average	Range	Day		Average number
				Average	Range	
I	53	11	1- 60	16	1- 80	6,900
II	73	52	22–100	64	30–134	7,400
III*	73	51	28- 80	64	40–105	8,740
IV	92	70	27–252	81	42–259	4,200
V	154	128	1–600	142	36–600	5,050

* Average of 9. One never learned to turn the cage.

TABLE 3

Giving the average number of days in revolving cages, age at death, total miles run, number of litters, days of reduced activity during reproduction and miles lost, the computed total miles and daily run

GROUP	AVERAGE DAYS IN CAGE	AVERAGE AGE AT DEATH	AVERAGE MILES RUN	REPRODUCTION			COMPUTED	
				Average number of litters	Average number days used	Average miles lost	Average total miles	Average miles daily
I	798	840	2,622	4 9	220	1,181	3,803	4 766
II	917	938	2,735	5 8	261	1,398	4,133	4 508
III	903	925	2,795	5 4	243	1,302	4,097	4 538
IV	840	862	2,546	2 7	121	651	3,197	3 806
V	894	920	2,683	1 8	81	433	3,110	3 479

these ages represent sexual maturity in these different groups will be discussed in a later paper. It is thus seen that diets rich in protein delayed the onset of spontaneous activity in almost a direct ratio to the per cent of protein used. As the diets increased in protein content the initiation for running became less.

When the activity had reached full swing it is seen that group III showed the greatest activity and was followed closely by groups II and I in order.

It is also noted that groups IV and V manifested the least activity. Even though there was an initial retardation in beginning activity in groups II and III they soon established a greater average activity than any of the other groups. This initial amount was gradually increased in each group as the animals became older.

In the first three columns of table 3 we have given for each group the average number of days in the revolving cages, the average age at death and the average total number of miles run. It is seen that group I had the least number of days in the revolving cages. This was due to being approximately 20 days older than the other groups when put in the cages and to a shorter life span. This shorter time in the revolving cages was partly compensated by the earlier starting of activity by this group (table 2).

If running activity is a measure of efficiency then the order of the value of the diets used from best to poorest as indicated by the total number of miles ran was III, II, V, I, and IV. There is, however, another element of functional activity which must be taken into consideration as it exerts a marked influence on the spontaneous activity of the female rat. It has been previously shown (Wang, 1923; Slonaker, 1925) that pregnancy and lactation greatly reduce the spontaneous running activity of the albino rat. The activity of animals which were averaging 10,000 revolutions daily dropped immediately after successful coitus to approximately 4000 and remained at that low rate for the 22 days of gestation and approximately the first 23 days of lactation. Since all the groups did not average the same number of litters and since those having few litters would have a greater number of days of normal maximal activity, we have made an attempt to compensate for this difference. This is shown in table 3 under the headings of "reproduction" and "computed." We have used 45 days as the interval of reduced activity for each litter born and 6000 revolutions as the average daily loss due to reproduction. From these data we were able to compute the average number of miles lost during the time by each group. This lost mileage was added to that actually run which gave the total mileage each group was capable of running had not reproduction intervened. These totals and the average daily run are given in the last two columns of table 3. These daily averages represent the possible daily activity for the entire time the animals were in the revolving cages. In table 2 we saw that the different groups became active at various intervals after being placed in the cages. If this is taken into consideration we find that the average number of days of activity for each group from I to V was 787, 865, 874, 792, and 792 days. This would make the average daily run during these days of activity 4.83, 4.78, 4.68, 4.04, and 3.92 miles respectively. This indicates also the order of efficiency of our diets if measured by the amount of daily work during the activity period. But when the total amount of work is considered we find the arrangement in

the computed mileage varying only a little from that of the miles actually run. The computed total miles run gives the efficiency of our diets from most efficient to least in the order of II, III, I, IV and V while that of actual run was from best to poorest was III, II, V, I, and IV. These results indicate that the maximal amount of activity in the rat would be obtained when it was fed on a diet containing between 14 and 18 per cent of protein. Since the spontaneous activity is a good index of the physical condition of the animal one might reasonably conclude that other physiological functions would be best performed on a similar diet. We have already shown in the first paper of this series, to which reference has already been made, that the order of maximal weights of both males and females was, from greatest to least, group III, II, I, IV, and V. This is in close correspondence with the results given above. It may also be noted that group II had the greatest average age at death and was closely followed by group III.

SUMMARY

When five groups of young female rats (I, II, III, IV, and V), were fed on diets containing 10, 14, 18, 22, and 24 per cent protein respectively the following results were found.

1. That the number of days intervening from the time they were placed in revolving cages until spontaneous running began was least in the group receiving the lowest per cent and in general increased as the protein content became richer.

2. That the order of magnitude of the different groups for the first few weeks of activity, from the highest to the lowest, was III, II, I, V, and IV.

3. That the total distance run, from greatest to least, was group III, 2795 miles; II, 2735; V, 2683; I, 2622; and IV, 2546.

4. That if allowance be made for activity lost incident to reproduction the order, from greatest to least, was group II, 4133 miles; III, 4097; I, 3803; IV, 3197; and V, 3110.

5. That a diet containing between 14 and 18 per cent protein is best suited to the albino rat for maximal spontaneous running.

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THE VISCOSITY OF THE BLOOD IN NARROW CAPILLARY TUBES

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Nearly one hundred years have passed since the French physician Poiseuille (1) took up for consideration the important problem of the resistance of the bloodstream in the narrow parts of the vascular system. As experimental difficulties arose with blood, his fundamental investigations were confined to experiments with water and different fluids in glass capillaries. He found, as is well known, that the time of efflux of a given volume of fluid is directly as the length of the tube, inversely as the difference of pressure at the two ends and inversely as the fourth power of the diameter.

During the following time the law of Poiseuille seems to have been generally accepted with regard to the blood also and the results of the first experimental studies by Ewald (2) and Benno Lewy (3) were, like those of many re-examinations, in accordance with the law in question. The now prevailing opinion is that the blood behaves as a real fluid in the vascular system with regard to its viscosity (see the survey by Neuschlosz, 4).

Against this opinion, however, objections have been made from a theoretical point of view. Von Kries (5) and Hürthle (6) have pointed out that the results of investigations of the viscosity of the blood in comparatively wide capillary tubes probably do not apply to the conditions in the narrower parts of the vascular system, whereby these authors especially seem to have had the true capillaries in view.

As far as we know, the only statement in the literature regarding the viscosity of the blood being altered in narrow capillary tubes is found in the paper of Denning and Watson (7). In the narrowest capillary employed in their researches, namely, one of a diameter of 0.3 mm., they observed *increased* values. This result is, however, certainly erroneous, for reasons that will be given below.

It is true that the viscosity of the blood has been earlier investigated in capillary tubes, where a divergence from the law of Poiseuille—corresponding to our own results—ought to appear. Thus Hess (8) has examined the viscosity of the same blood in capillaries of a diameter of 0.239 and 0.126 mm. respectively under the same conditions. A calculation on the basis

of the values, he found, gives a relative viscosity in the narrower one, which is only 89 per cent of that in the wider one—a fact which the author himself seems to have overlooked.

The present investigation deals with the problem of the influence of the diameter of the capillary tube upon the viscosity of the blood and pays special attention to the conditions in capillary tubes which are as narrow as possible.

The blood was obtained from one of us through venepunction. To prevent coagulation 10 cc. of blood were mixed (in the syringe) with 0.5 cc. of a concentrated sodium citrate solution, an addition which may have some influence upon the viscosity of the blood, but which is of no importance in our experiments which were only intended to make a comparison between the values obtained in capillary tubes of different diameter.

Many investigators have used viscosimeters of Ostwald's type, where the blood from a reservoir is pressed by its own weight through a vertical capillary. This apparatus has, however, a great source of error. The red corpuscles have time to settle in the reservoir, during the experiment. The consequence of this will be that the concentration of the corpuscle suspension entering the capillary will change during different phases of the streaming. This complication will have a great influence in the direction of increased time of efflux, especially when sinking speed of the red cells is large as is the case in human blood under many pathological conditions, and also in horse blood. This fact certainly explains the above mentioned result of Denning and Watson, who found the viscosity of horse blood increased in narrow tubes.

Also in viscosimeters constructed after the principle of the apparatus of Hess even normal human blood will lose its homogeneous condition through the sedimentation of the red cells, when the time of the determination is prolonged, as will be the case when using such narrow capillary tubes as in our experiments.

The researches here in question have been performed with an apparatus arranged in the following way.

Glass capillary tubes[1] of length and diameter as given below, were at both ends put together with wider cylindrical glass tubes, one of which served as reservoir for the fluid to be determined, the other being used as receptacle of the fluid which had passed the capillary. With its receptacle end this "viscosimeter" was connected by a short rubber tube with the movable part of an airtight hollow glass bearing. With a longer rubber tube the fixed part of the glass bearing was joined with a manometer of Hg and further with a big glass bottle, protected against changes of temperature and evacuated so as to obtain the desired pressure. As will be seen from

[1] The capillaries were prepared from machine-worked glass tubes specially ordered from Glasfabrik Sophienhütte, Ilmenau, Thüringen, Germany.

the figure 1, the viscosimeter was brought into a somewhat wider glass tube, put through the side walls of a thermostate (38°C.) quite close to its anterior glass wall. To prevent the sedimentation of the red corpuscles, the viscosimeter was kept in a rotary motion within the glass tube of the thermostate. The speed of the rotation was about one turn per second. The displacement of the meniscus of the fluid in the receptacle glass tube for a certain number of millimeters, was determined by a Zeiss measuring microscope placed before the glass wall of the thermostate and the time was measured with a stop watch.

Regarding the pressure used in our experiments, the following may be remarked. More than twenty years ago, Hess (9) found that the product of the time of efflux and the difference of pressure at the two ends of the capillary tube is increased, when too low pressures are used. This result, confirmed by Rothmann (10) and Rothlin (11) and, as a matter of fact forming a divergence from the law of Poiseuille, was not considered to have any influence upon the streaming resistance of the blood in the vascular

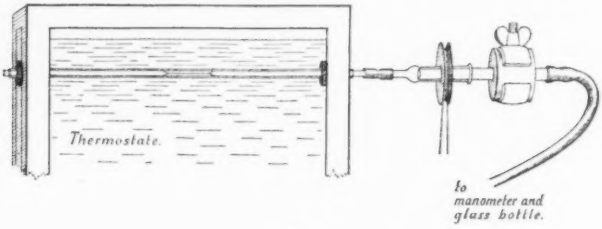

Fig. 1

system, but to be of great importance for the technics of measurement of blood viscosity. In this connection, it may be remarked that the authors mentioned apparently overlooked the fact that the length of the capillary tube has as great an influence as the difference of pressure at the two ends but in the opposite direction; in other words, it is the fall of pressure per unit of length of the capillary tube which is decisive with regard to the point where the divergence in question occurs. As we were anxious to investigate the influence of the diameter of the capillary tube upon the viscosity of the blood above the critical fall of pressure per unit of length of the same, certain preparatory investigations were necessary, the result of which may here be omitted. We state only that the pressure of 100 mm. Hg, which has been used in these researches with regard to all capillaries employed, renders the experiments we made far above the critical point in question.

The relative viscosity of the blood was as usual obtained by dividing the figure representing the time of efflux in seconds for a given volume of blood with the analogous figure for water.

It is important to remark the values obtained with water in the different capillary tubes with regard to the relation between length of the tube, difference of pressure at the two ends, diameter of the same and time of efflux for a given volume agreed very well with the law of Poiseuille.

RESULTS. Four representative series of experiments are shown in the following tables. The corresponding curves are given in diagram figure 2.

LENGTH OF THE TUBE	DIAMETER	RELATIVE VISCOSITY
Series 1. Blood from T. L. Relative viscosity of the plasma 1.63		
mm.	*mm.*	
126.5	0.505	4.60
121.0	0.311	4.54
45.6	0.175	4.22
50.0	0.120	3.98
32.6	0.081	3.65
11.8	0.065	3.38
11.8	0.047	2.95
12.6	0.040	2.79
Series 2. Blood from T. L. Relative viscosity of the plasma 1.65		
122.5	0.277	4.96
45.6	0.175	4.72
50.0	0.120	4.30
11.8	0.047	3.15
Series 3. Blood from R. F. Relative viscosity of the plasma 1.60		
—	0.460	5.21
100.0	0.277	5.10
85.0	0.105	4.28
—	0.052	3.52
Series 4. Blood from T. L. Relative viscosity of the plasma 1.72		
In order to investigate a blood specimen of higher viscosity, the blood was centrifuged and a part of the plasma taken away.		
122.5	0.277	6.80
45.6	0.175	6.28
50.0	0.120	5.59
27.9	0.081	5.08
7.0	0.065	4.71
11.8	0.047	4.28

DISCUSSION. As will be seen from the diagram, the viscosity of human blood is far from being a constant quantity, but changes with the diameter of the capillary tube. These growing narrower, the viscosity begins to decrease in capillaries slightly wider than about 0.3 mm. and is subse-

quently more and more reduced, the lowest values being found in the narrowest capillaries used, namely, those of a diameter of about 0.04 mm. It was impossible to press the blood through still narrower tubes and therefore we could not continue the examination in this direction. But from the curves obtained, the viscosity seems to be further strongly reduced with reduced diameter of the tubes. Very likely in a capillary tube of a diameter, for instance, of 0.03 mm., the values are only about 50 per cent of those obtained in a capillary tube of a diameter of 0.3 mm. There seems to be no reason why the viscosity of the blood in still narrower tubes may not

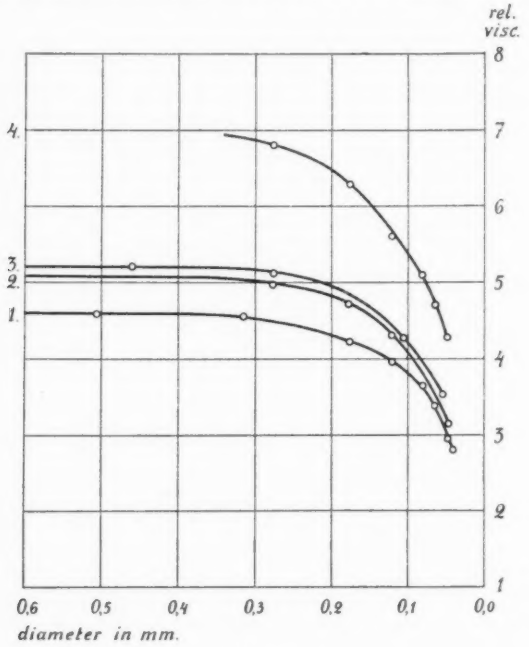

Fig. 2

come very close to or perhaps coincide with the viscosity of the plasma. *The law of Poiseuille does not apply to the flow of blood in capillary tubes of a diameter below about 0.3 mm.*

This fact has an important bearing upon the technics of measurement of blood viscosity. Contrary to the common practice in researches of this kind it is necessary to state the diameter of the capillary tubes, with which a certain value has been obtained. Our experiments show, for instance, that two different blood specimens (curves 3 and 4 in the diagram) give the same relative viscosity of 4.28 in capillary tubes of 0.105 and 0.047 mm.

diameter, though these blood specimens examined in capillary tubes of 0.3 mm. diameter or wider ones, i.e., within the range where the law of Poiseuille is still valid, show very different viscosity values.

But the result of these researches might be of interest also from an hemodynamic point of view. Our knowledge of the blood pressure at different parts of the vascular circuit points very distinctly to the chief resistance in the vasular system as being situated in the arterioles (Starling, 12). May be that what is called arterioles includes somewhat larger vessels, but certainly the greatest part of the resistance to the flow of blood is to be found in those parts of the arterial system that have a diameter from about 0.3 mm. down to the smallest arteries, from which the capillaries themselves ramify. If the viscosity of the blood like that of a fluid were a constant quantity independent of the diameter of the tube, the resistance ought, ceteris paribus, to increase with the fourth power of the diameter of the vessel. The result of our experiment in glass capillary tubes indicates, however, that that part of the resistance in the narrow vessels of the arterial system, which is due to the viscosity of the blood, is far less than has hitherto been expected. Thus the peculiar viscosity of the blood allows the heart to drive a given volume of blood through the arterioles at a much lower pressure than would be the case if the blood behaved as a fluid.

It is not necessary to point out that analogous conditions occur in vessels of corresponding width belonging to the venous sytem. The result of this investigation is not able to elucidate the state of affairs in the capillaries themselves, where the diameter of the corpuscles and the vessel coincide.

As to the physical explanation of the anomalous viscosity of the blood, here pointed out, it is certainly dependent on its being a suspension. The comparatively great viscosity of the blood is to a very high degree due to the suspended red corpuscles. The relative viscosity of the plasma of normal human blood is about 1.6. With addition of red corpuscles, the viscosity increases and reaches with the relative amount of red cells, characteristic for normal human blood, a value of about 4.5 to 5 (the viscosity determined in capillary tubes of a diameter above 0.3 mm.).

One of us (Fåhræus, 13) has recently stated that the blood streaming through a capillary tube of small diameter undergoes a change with regard to its composition. As is well known, the red corpuscles are transported in the fast axial stream, the slow marginal stream consisting only of plasma. The fact that the average speed of the red cells is thus greater than that of the plasma particles has the consequence that the amount of corpuscles in relation to the amount of plasma will be diminished; in other words, the corpuscle suspension will be diluted while streaming through a narrow capillary tube. The following table shows the average velocity of the plasma and the corpuscles and the composition of the blood, while streaming through capillary tubes of different diameter.

DIAMETER OF THE TUBES	COMPOSITION OF THE BLOOD		AVERAGE VELOCITY OF THE ERYTHROCYTES, THAT OF THE PLASMA = 100
	Erythrocytes	Plasma	
mm.	*per cent*	*per cent*	
1.100	40.5	59.5	100
0.750	40.1	59.9	101
0.450	39.8	60.2	103
0.250	39.2	60.8	106
0.095	33.6	66.4	135
0.050	28.0	72.0	175

As will be seen the relative velocity of the corpuscles and the accompanying dilution of the corpuscle suspension begin to appear in tubes of a diameter of about 0.3 mm. and rapidly increase in narrower tubes. It seems very probable that it is this dilution of the corpuscle suspension which causes the anomaly of the blood viscosity, shown by these experiments.

SUMMARY

The viscosity of the blood is not a constant quantity, but dependent on the diameter of the tube. Below a critical point at a diameter of about 0.3 mm. the viscosity decreases strongly with reduced diameter of the tube. As a consequence the resistance to the flow of blood in the arterioles (and in the small veins) is considerably less than would be the case if the streaming of the blood followed the law of Poiseuille, *i.e.*, behaved as a fluid with regard to its viscosity.

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THE WATER EXCHANGES OF FROGS WITH AND WITHOUT SKIN

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Water regularly enters the bodies of frogs through the skin. It was found (Adolph, 1925, 1927) that its passage was strongly inhibited when the frogs were in certain media, such as pure water, as compared to its passage when the frogs were in other media, such as hypertonic solutions of sodium chloride. Observations of the passage of water through skin isolated from the body indicated that a part of the peculiar control of its passage resided within the skin.

The precise measurement of this control was obviously to be obtained by comparing with intact frogs, frogs from which the skin had been removed. These experiments are now to be reported; some of the results were previously indicated in abstract (Adolph, 1929). In addition, the effects of anesthesia, of pithing, and of trauma upon the initial exchanges of water when the frogs were placed in various solutions are to be evaluated.

METHODS. The observations here described are limited to the species *Rana pipiens*. The amounts of water exchanged by frogs were measured by weighing the individuals at frequent intervals. It seems important to describe the exact methods used in weighing, for surprisingly concordant results have been obtained when a certain technique is rigidly followed. Frogs are weighed singly upon a triple-beam balance to 0.01 gram, or in groups of two to four individuals upon a trip balance to 0.1 gram. They are lifted individually from the medium, their abdomens are slightly compressed to expel urine, and they are placed in an aluminum cup that has a screen cover which can be latched. After the weight is taken, the frogs are lifted from the cup and replaced in the solution; the cup with the water which has drained from the frogs is then weighed as the tare. This procedure has the incidental advantage of not allowing the observer to know the weight of the animals at the time of determining it.

At the beginning of each experiment the frog is weighed twice before anything is done to it. These two weighings have on the average the least agreement of any duplicate weighings made during the experiment; their deviation from their mean is on the average 0.30 per cent of the body weight.

Anesthesia was administered by placing frogs into water in which the anesthetic was dissolved. Urethane was used as a 0.8 per cent solution; chloretone as a solution one-twelfth of the concentration saturated at 27°C. Either of these solutions obliterates all response to mechanical stimuli within one hour, smaller individuals becoming narcotized sooner than larger ones.

To remove the skin from a frog, the skin is pierced with scissors just behind the lower lip. From this incision the skin is cut around the head behind the lower lip and just behind the ears and eyes. Some care is taken not to cut the large veins just dorsal to the corners of the jaws. The skin is reflected ventrally, the ligaments holding it to the throat are cut, and then the whole skin is slipped off inside-out. The only skin that remains is on the forehead, which is a constant proportion of the whole skin.

The skinless frog is now weighed and immediately, noting the exact time, is put into a solution, just as was previously done with intact frogs. The skin as soon as removed is put into a weighing bottle, and the bottle is weighed with and again without the skin in it. The sum of the weights of the flayed frog plus the skin averaged 5.17 per cent below the weight of the intact frog. This loss upon dividing the body into skin and remainder was mainly due to loss of blood and lymph, but a small part was due to the removal of more water from the outer surface of the frog during the handling incident to flaying.

Skinless frogs in NaCl solutions. Individuals from which the skin was removed were studied either singly, each one being placed in 200 cc. of solution, or in groups of three, which were placed in 500 cc. of solution. Individuals which were weighed only initially and at the end of 30 minutes showed the same average changes as those weighed three to eight times within 30 minutes, indicating that handling and repeated temporary removal from the bulk of the solution had no measurable influence.

Upon being placed, directly after removal of the skin, in various concentrations of sodium chloride, water was progressively taken up by the frogs. The changes of body weight in six concentrations, that were chosen at 0.04 M intervals, are plotted for a single experiment in figure 1. The changes are plotted against the square root of the time elapsed; for initially they were aways in linear proportion wherever the concentration of sodium chloride was hypotonic. In the higher concentrations of sodium chloride the rate after a few minutes was different from that indicated by the first one or two weighings. This raised the question whether the first or the later rates were most characteristic of the passage of water into the frogs. To decide this point, all of the data reported in the present paper were analysed in three ways. First, the amounts of change at 30 minutes after immersion in the solution were compared. Second, the rates as indicated by the first and second weighings were compared. Finally, the

rates indicated by the slope of the line connecting points later in the experiment, but always within the first hour, were compared. The last comparison proved to be the most reproducible, though the first was almost as reliable. While the rates of passage of water are spoken of as initial rates, it should be clear that the rates do not apply in the first 5 to 15 minutes in those experiments where the frogs lost weight.

Exact comparisons of the rates at which water is taken up by frogs require that the volumes of water taken up be referred to the body surface. This is evident from the fact that water must diffuse first into the skin, as well as from the data previously collected (Adolph, 1927) on the relation between body weight and rate of change of weight in sodium chloride solu-

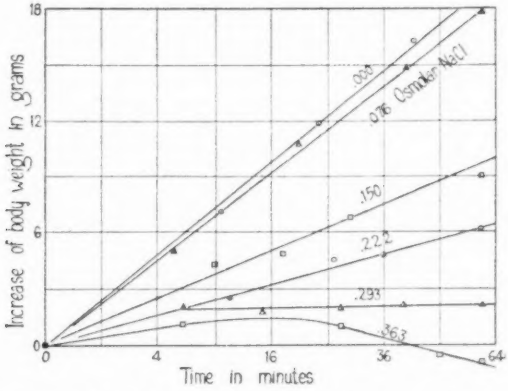

Fig. 1. Time course of the changes of weight undergone by skinless frogs placed in sodium chloride solutions of various concentrations. The numerals represent osmolar concentrations. Each line represents a group of three frogs that were anesthetized with chloretone before their skins were removed; the six groups were studied simultaneously. Temperature 19°C.

tions. A few measurements of the area of freshly isolated frog skins indicated approximately that body surface in cm.^{2} equals $8 \times$ (body weight in grams)$^{2/3}$; this equation was used for estimating the body surface in all experiments. The surface of the skinless body was arbitrarily taken to be equal to that of the skin itself.

The data of each experiment are thus reduced to cm.^{3} of water diffusing through one cm.^{2} of body surface in the square root of the number of minutes that have elapsed after transfer of the individual to the salt solution in question.

The initial rates of water intake by the skinless frogs in each of the several concentrations of sodium chloride are compared in figure 2. The average points for skinless frogs in solutions of pure sodium chloride fall closely along a straight line, and the best fitting line has been drawn in.

In the first series of experiments the frogs were immersed for one-half to two hours in the anesthetic solution, at the end of which time the skins were removed. Then they were placed in one or another of the media to be tested, which did not contain anesthetic. In these media they sometimes recovered from anesthesia and any other effects of operation, though they never became spontaneously very active because of the absence of sensory stimuli.

The influence of the anesthetic may be judged to be non-specific from the fact that the two solutions used, urethane and chloretone, gave similar quantitative results in skinless frogs, as was proven for the concentration 0.076 osmolar sodium chloride.

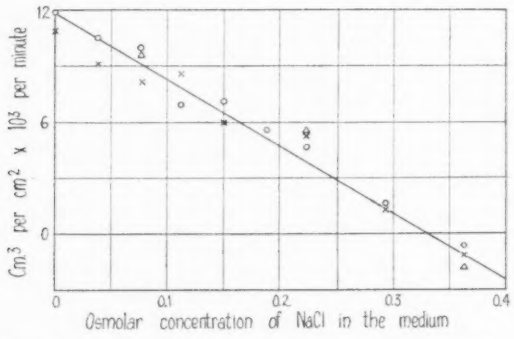

Fig. 2. Relation of initial intake of water by skinless frogs to the concentration of sodium chloride in the medium. Each triangle represents the mean of 3 to 11 determinations upon a group of three individuals anesthetized with either urethane or chloretone. Each cross represents the mean of 2 to 4 determinations upon single pithed individuals. Each circle represents the mean of 4 to 6 determinations upon single normal individuals. Temperatures 19° to 30°C.

A second series of experiments was done with skinless frogs that were pithed, both brain and spinal cord being destroyed. The mean results so obtained are shown in figure 2. The best straight line correlating these points does not differ significantly from the best line for anesthetized frogs.

At this point in the experiments intact frogs that were anesthetized and pithed were compared with normal intact frogs. The rates of osmosis into these individuals were markedly different from those into normal intact frogs, as will be shown later. Were the skinless frogs to be compared with the intact ones similarly anesthesized or pithed, or with the normal ones? A third series of tests was therefore made upon frogs from which the skin was removed without anesthesia, the results of

which are also shown in figure 2. It turns out that the initial rates of osmosis are, within the limits of accuracy of the determinations, the same whether the skinless frogs be normal, anesthetized, or pithed.

Skinless frogs gain weight or lose weight at initial rates that are linearly correlated with the concentration of sodium chloride in the medium, or with the osmotic pressure of this salt in the medium. They gain weight in all concentrations of sodium chloride up to about 0.33 osmolar. The normal total concentration of the blood and lymph of Rana pipiens is 0.235 osmolar and of the muscles is 0.325 osmolar (Adolph, 1927). Obviously water is moving into the body of the frog even when it is immersed

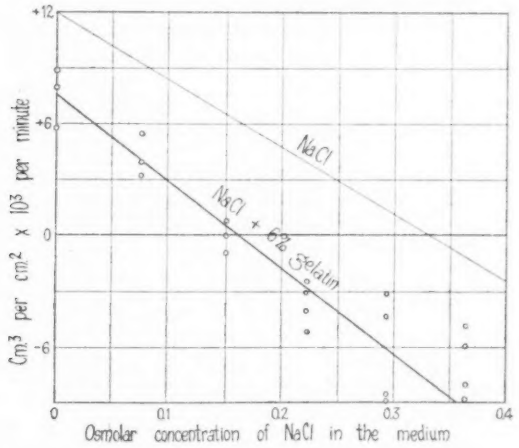

Fig. 3. Relation of initial intake of water by normal frogs whose skins were newly removed to the concentration of sodium chloride in the medium, which always contained 6 per cent of gelatin. Each point represents one determination upon one individual. The upper line is the average curve of figure 2, placed here for comparison. Temperatures 25°C. to 30°C.

in a concentration of salt which is stronger than the total concentration of the blood or lymph. No correction is required for the rate of urine formation in such rapid exchanges as are here measured.

SKINLESS FROGS IN NACL + GELATIN SOLUTIONS. In order that the passage of water be actually influenced in accordance with the relative osmotic pressures which prevail on either side of the frog's surface, it seemed possible that colloidal constituents are required in the medium. For this reason experiments were performed in a similar manner as before, but with 6 per cent of gelatin added to the various concentrations of sodium chloride. The gelatin was dissolved in tap water at 50° to 60°, cooled to 27°C., and kept at that temperature in a highly fluid state.

In these solutions, too, skinless frogs gained or lost body weight initially in proportion to the square root of the time. During immersion in the gelatin solutions, the uptake of water was likewise linearly correlated with the dilution of sodium chloride, as is shown in figure 3. The slope of this straight line is significantly greater than the slope of the line for the frogs in pure sodium chloride solutions.

But the line for gelatin-containing solutions is displaced very far from that without gelatin, so that the skinless frogs lose weight in all concentrations of sodium chloride above 0.16 osmolar. To make sure that such a difference was due to nothing but the solution, many experiments with and without gelatin were run simultaneously. In every experiment the difference was highly significant.

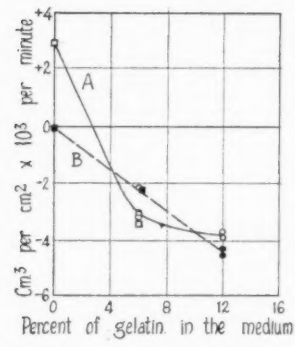

Fig. 4. Relation of initial intake of water to the concentration of gelatin in the medium that always contained 0.235 osmolar sodium chloride. *A*, anesthetized skinless frogs; *B*, normal intact frogs. Each circle represents a determination upon a group of three individuals at 27°; the squares are average points read off from figures 2, 3, and 7.

Whereas in solutions of pure sodium chloride the flayed frogs gained weight in all concentrations up to 0.33 osmolar, when 6 per cent of gelatin was present the body weight remained constant in 0.16 osmolar. The first value is much above the total concentration of the frog's blood as measured by its freezing point, the latter value much below it. With about 3 per cent concentration of gelatin, 0.235 osmolar sodium chloride is the point of no water exchange; if any forces other than osmotic pressures exist, they are apparently then equal inside and outside the frog.

The effect of a still higher concentration of gelatin in the medium was studied by choosing for further experiments that concentration of sodium chloride (0.235 osmolar) that has the same osmotic pressure as whole frog blood. Figure 4 shows that osmosis into skinless frogs was not greatly influenced by raising the concentration of gelatin in this medium above 6 per cent.

The effect of gelatin was not merely to retard osmosis, for its influence was not proportional to the movement of water that would occur in its absence. In the concentrations of sodium chloride between 0.16 and 0.33 osmolar (fig. 3), the direction of movement was actually reversed by the presence of 6 per cent of gelatin.

It may be supposed that some of the difference in rates of osmosis is due to the osmotic pressure exerted by the gelatin itself. Measurements of

this pressure made by Lillie (1907) across wet collodion membranes yielded the values for a 1.25 per cent solution of gelatin of 6.7 mm. of mercury against water, and 2.9 mm. of mercury against 0.02 to 0.08 osmolar NaCl solution. The highest value of 6.7 mm. indictates that a 6 per cent solution corresponds to a concentration of less than 0.002 osmolar gelatin, which is an almost imperceptible magnitude on the scale of figure 3. The osmotic pressure of the gelatin is somewhat higher than

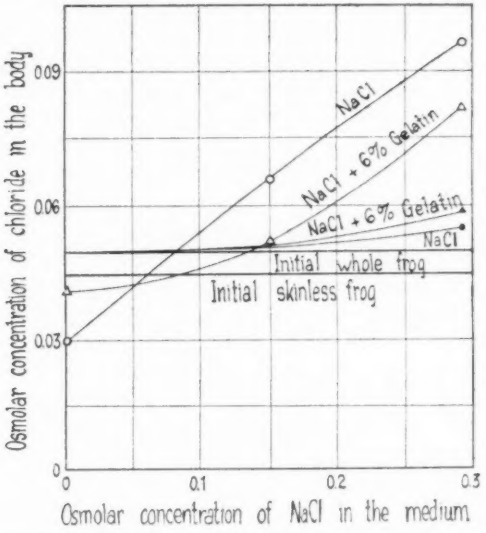

Fig. 5. Chloride concentrations in the bodies of frogs which were immersed in various concentrations of sodium chloride for 30 minutes. The two upper curves represent skinless frogs immersed in the solutions, and the two lower curves represent intact frogs immersed in the solutions but skinned before being analysed. The "initial" or control frogs were analysed directly as taken from tap water. Each point is the mean of 2 to 5 determinations on single individuals kept at 27°. The osmolar concentrations are calculated from the chloride found per unit of body weight, on the assumption that the chloride represented a solution equal in osmotic pressure to a solution of NaCl of the same weight.

this in acid and alkaline solutions, as Lillie (1907) found. Colorimetric estimations of pH in the gelatin solutions as used here showed that the hydrogen ion concentration was always between pH 5.5 and pH 7.0; the presence of the frogs in the solutions did not change the pH appreciably.

The measurements of the osmotic pressure of gelatin contained in collodion sacks made by Lillie (1907) are confirmed by Loeb (1922), Northrop and Kunitz (1926), and Adair and Callow (1930). In figure 3 it is seen that the attraction exerted by 6 per cent of gelatin is roughly

equivalent to the attraction exerted by 0.17 osmolar sodium chloride. Hence the osmotic effect exerted by the gelatin is 85 times that exerted by sodium chloride of equal osmotic pressure. This illustrates the well-recognized fact that a salt solution is osmotically a substitute for a body fluid only when a colloidal material of about this concentration is dissolved in it.

CHLORIDE EXCHANGES OF SKINLESS FROGS. An obvious hypothesis concerning the effect of gelatin upon the water exchanges of flayed frogs was that the gelatin affects the movement of the water not directly, but through solutes. If some salt is combined with the gelatin, or if the tissues exposed to the solution are rendered by the gelatin less permeable to salt, then less water might effectively be attracted by the osmotic pressure of the salt. The chloride contents of whole frogs and of skinless frogs were determined by the convenient procedure of Van Slyke (1923).

Individual frogs were skinned and placed in various concentrations of sodium chloride, with or without 6 per cent of gelatin, for 30 minutes, at the end of which time the entire skinless bodies were analysed for chloride. The results of the analyses are shown in figure 5. The concentration of chloride is referred not to the amount of water present in the body, but to the total weight of the flayed frog at the beginning of the period of immersion in the solution. This initial weight was chosen in place of the final weight in order to free the results as much as possible from the effects of the simultaneous exchanges of water. But in any case the changes of chloride content are so large as to give the same essential result irrespective of the exact basis of calculation.

In every concentration of sodium chloride, less chloride was given up or taken up by the skinless frog's body when gelatin was present. In zero concentration, chloride was held more tenaciously by the frog when gelatin was present in the medium, yet water was not absorbed by the frog so rapidly as when gelatin was absent. At the concentration 0.15 osmolar, salt and water entered the body proportionately to one another. But at 0.29 osmolar NaCl + gelatin, salt entered the body while water left it. There is no correlation of the relative rates of penetration of water and of chloride which would hold for the various concentrations of the medium both in the presence and in the absence of gelatin, as is shown by figure 6.

For comparison, determinations of the chloride content of the body minus the skin were made upon frogs which were immersed whole and intact for 30 minutes in 0.16 M NaCl, and in 0.16 M NaCl + 6 per cent gelatin. These results illustrate the important rôle of the skin in retarding the exchanges of chloride. First it should be noted that the skin of the frog is very rich in chloride, the average concentration in 22 normal skins (average weight 4.06 grams) being 0.0471 M or 0.0901 osmolar. This is greater than the chloride content of any other region or tissue of the frog.

The two "initial" mean values of figure 5 were determined upon the same five individuals, the skins being analysed separately and their content added to the content of the skinless bodies of the "whole" individuals.

In 0.16 M NaCl the whole frogs gained chloride, but only slowly in comparison with the skinless individuals. But significantly more was gained when gelatin was present in the medium than when it was absent. The shape of the interpolated curves for the total chloride concentration of whole frogs, drawn in figure 5, is that found previously for frogs minus the hind legs (Adolph, 1927); the points newly determined at 0.16 M serve to locate the curve upon the ordinates.

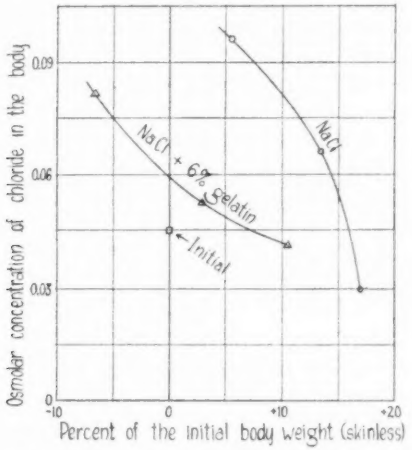

Fig. 6. Changes of chloride content of skinless frogs in relation to changes of water content, in the first 30 minutes of immersion in the various solutions tested in figure 5.

It might be suggested that what is required is a knowledge of the amounts of chloride and particularly of sodium combined with and left free by the gelatin present. The effects of serum protein (Bugarszky and Tangl, 1898) and of gelatin (Palmer, Atchley and Loeb, 1921) upon the electrical conductivity of salt solutions are known, but offer little information useful in the present connection. It is likely that the greater slope (fig. 3) of the curve of water intake for skinless frogs when gelatin is present is correlated with the lesser chloride exchange when gelatin is present. But it remains true that the effect of gelatin was almost as great where no salt was added to the medium as where a high concentration of salt was present. That the effect was not fully as great is evidence of some influence either of or upon the salt.

Any diffusion of solute sets up local conditions such as are manifested as diffusion potentials and frictional potentials. These are known to influence in turn the passage of water, but are usually very small in magnitude. These forces are not predictable, since among other factors they depend upon "zeta" potentials, which cannot be measured in a membrane system.

Besides the above factors, the important factor remaining is the larger permeability to sodium and chloride than to gelatin on the part of the tissues. If the tissues allow the salt to pass readily into and out of them, then the salt is unable to exert its full osmotic pressure. This factor can be illustrated by experiments carried out with artificial membranes.

When collodion sacks were made by soaking the freshly-formed sack in water, according to the method of manufacture ordinarily used, it required a solution of sodium chloride of at least 0.2 osmolar in concentration to cause measurable amounts of water to flow through the membrane, under zero hydrostatic and capillary pressures. Through the same sacks a 6 per cent or a 1 per cent solution of gelatin caused a rapid flow of water, and this water flowed against a hydrostatic pressure almost equal to the osmotic pressure exerted by the gelatin.

But when dried collodion sacks were made, by completely evaporating the ether and alcohol solvents before allowing the collodion to touch water, and later soaking the membrane in alcohol according to the method of Brown (1922), then it required a solution of only 0.002 osmolar sodium chloride to cause water to flow through the membrane.

This experiment constitutes a model of the osmosis into frog tissues. If the tissues were truly semi-permeable they would swell enormously faster than they do in any hypotonic solution of sodium chloride and shrink faster in any hypertonic solution. In other words, the slope of the line in figure 3 is a function of the permeability of the tissues to the salt in which the tissues are immersed. If instead of varying the sodium chloride concentration, as in figure 3, the concentration of gelatin was varied, as in figure 4, then the osmotic effect of the gelatin manifested itself in a slope so great as to be almost vertical on one of the charts of osmolar concentrations. The present "plasmolysis" experiments with sodium chloride resemble most others of the kind in demonstrating the ready penetration of the tissue by the solute.

There is no evidence that forces of hydration, colloidal swelling, or water-binding are manifested when frogs are immersed in gelatin solutions.

Living frogs from which the skin has been removed, therefore, exchange water in linear proportion to the concentration of sodium chloride in the medium. With a chosen concentration of gelatin as well as of salt present, they behave completely like ideal osmometers in that when the osmotic pressure inside the body is equal to that outside there is no exchange. The influence of gelatin differs from that of sodium chloride because the

latter solute penetrates the tissues very rapidly while the former does not. Part of the influence of gelatin upon osmosis is exerted through its retardation of the exchanges of salt.

INTACT FROGS IN NaCl SOLUTIONS. The water exchanges of whole frogs upon transfer from pure water to various concentrations of sodium chloride have been previously measured (Adolph, 1927). The average curve for the initial rates, derived from a large number of measurements, is the "normal" curve of figure 7.

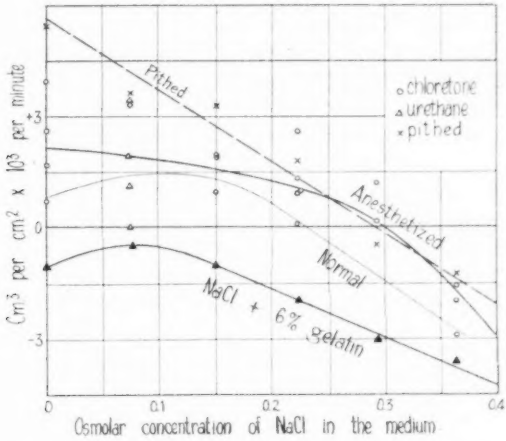

Fig. 7. Relation of initial intake of water by intact frogs to the concentration of sodium chloride in the medium. The open points represent determinations upon groups of three anesthetized individuals. Each cross is the mean of 3 to 7 determinations upon single pithed individuals. The light line is the average curve for normal intact frogs taken from Adolph (1927) and confirmed by the control determinations of the present experiments. Each solid triangle represents the mean of 4 to 7 determinations upon groups of three normal individuals that were immersed in salt solutions to which 6 per cent of gelatin was added. Temperature 27°C.

The effect of anesthesia was tested upon intact frogs. When taken from water and placed in urethane or chloretone solutions, water was taken up in significant amounts. The mean gain in one hour was 2.0 per cent of the body weight (30 determinations, 90 frogs), and 5.5 per cent in two hours (10 determinations, 30 frogs); thereafter the rate of gain decreased. The curve of weight increase was the same for both anesthetics. When the same frogs were taken from the anesthetic solutions and placed in tapwater there was no immediate decrease of body weight but usually an increase.

When intact frogs, fully anesthetized, were placed in various concentrations of sodium chloride, they exchanged water at rates that were sig-

nificantly different from those for unanesthetized intact frogs. The salt solutions in no experiments contained anesthetic. At all concentrations more water was gained or less water was lost, so that the curve for rate of osmosis (fig. 7) was above the curve for unanesthetized frogs. The anesthetized frogs, in contrast with the normal and pithed frogs, gave very irregular changes of weight in relation to time. It is possible that all of these effects of anesthesia upon intact frogs are due to the influence of the anesthesia persisting to varying degrees after the frogs were removed from the anesthetic solution; since a part of the body weight changes are not reversed when the anesthetic is removed.

If the results on anesthetized skinless frogs had been judged in the light of the results on anesthetized intact frogs instead of the results on unanesthetized skinless frogs, a slightly different and seemingly erroneous base line would have been used in estimating the effect of anesthesia plus flaying.

The general influence of anesthetics upon permeability has been much discussed; but the investigations that have to do directly with the rate of passage of water under their influence are limited to the diverse experiments of Loeb (1912), Winterstein (1916), Lillie (1918) and Heilbrunn (1925). Loeb demonstrated that Fundulus embryos when placed in hypertonic sea water shrank more rapidly to the point of sinking if in the presence of various alcohols. Lillie and Heilbrunn observed that sea-urchin eggs when placed in hypertonic sea water shrank more slowly and when placed in hypotonic sea water swelled more rapidly if in the presence of ether; and Winterstein and Heilbrunn found that freshly isolated frog muscles when placed in distilled water swelled more slowly if in the presence of a narcotic. It was obviously impossible to gain from these various observations upon diverse living materials any notion as to what would happen to the rate of osmosis into a frog if anesthetics were present.

The influence of pithing both the brain and the spinal cord upon the initial rates of osmosis was studied, to see whether the initial rates corresponded to the reported increase of water retention of frogs so treated, (Pohle, 1920, and others). Intact pithed frogs took up water at initial *rates that were linearly correlated with the concentration of the medium* (fig. 7). This result contrasts these frogs with the normal and the anesthetized intact frogs. The effect of the pithing is immediate, for when a frog is merely taken from pure water with which it is in osmotic equilibrium, is pithed, and returned to the same water, it begins to swell at an enormous rate.

Pithing the intact frog may be regarded as the physiological equivalent of removing the skin. The peculiar ability that enables the skin to oppose the rapid entrance of water from hypotonic media is lost. This opposition apparently resides in the skin, yet it vanishes as soon as the central nervous

system is destroyed. The orientation known to be exhibited by skin isolated from pithed frogs may be only a small part of that exhibited by the skin in the normal frog. Or possibly the skin may be freed by isolation from an inhibition that previously appears at pithing. Pithed frogs do not respire through the lungs, and it is possible, though not likely, that eventually anaerobic changes modify the water-attracting forces of the frog's tissues.

If the results on pithed skinless frogs had been judged by comparing them with the results on pithed intact frogs, it would have been concluded that the skin made little or no difference in the water exchanges of frogs.

The effect of gelatin in the medium was compared to the effect of sodium chloride upon intact frogs. A series of determinations was made with 6 per cent of gelatin added to the various concentrations of sodium chloride. The frogs were always acclimatized to tap water at 27° C. before the experiment started. In all but three of the 31 determinations there was a loss of weight upon transfer from the water to a gelatin solution; the average points are shown in figure 7. Even without salt in the medium a significant decrease of water content initially occurred. All the tests in gelatin were controlled by simultaneous weighings of frogs kept in tap water at 27°.

These results were extended by a series of experiments in which the intact frogs were transferred back and forth between a certain concentration of sodium chloride with and without 6 per cent of gelatin. The changes of weight within one hour were usually not great, which made the accuracy of evaluation relatively small. The majority of determinations confirmed, however, both the directions and the mean amounts of exchange that were to be expected from the positions of the two curves in figure 7.

The effect of the concentration of gelatin was further illustrated by comparison at the isotonic concentration of sodium chloride in the medium (fig. 4). So far as the data go, the effect of gelatin upon initial exchanges of water by intact frogs is proportional to its concentration.

When sodium chloride was omitted from the medium the effect of gelatin was not marked. In various concentrations of it, water was drawn from the intact frogs at extremely slow rates as compared with the rates when the salt was also present. The exact importance of this fact needs further elucidation.

THE INFLUENCE OF TRAUMA. A frog from which the skin has been removed has suffered more than privation of a particular tissue. One of the chief injuries is hemorrhage. This was sufficient in a few instances to cause the circulation to fail in an hour or two, but in nearly all the experiments it was not sufficient to influence the experimental animals observably. The operation does, however, leave the circulation incomplete, for the body has been robbed of about 15 per cent of its "periphery." The ana-

tomical relationships are somewhat new, since tissues and blood vessels which normally border on the subdermal lymph spaces are now exposed to the medium. The minute blood vessels at the surface of muscles and connective tissues can be seen to dilate greatly.

Removal of the skin likewise exposes nervous tissues, especially broken nerves trunks that normally serve the skin. The effects of trauma to these nerves upon the water balance of intact frogs were investigated by Pohle (1920), who found no departure from normal, in spite of the perforation of the skin during the operation. The central nervous system has been more extensively studied; Pohle (1920) and Jungmann (1923) found that frogs retained much more water than they normally do as soon as pithing or sympathectomy was performed, though Brugsch (1928) could find no influence of pithing. The significant results, however, point to the hypophysis as the essential organ whose function is interfered with in these experiments (Pohle, 1920; Jungmann, 1923; Tschernikoff, 1926). It has not been proven, therefore, that trauma to peripheral nerves has any influence upon water regulation of the frog. So far as experience indicates, a skinless frog placed in any of the media studied reacts osmotically to it just as "normally" as an intact frog. Only upon such a basis can the regularity and consistency of the results be accounted for.

Skinless frogs usually survive 4 to 8 hours, though some survive as long as 3 days if kept in Ringer's solution or sodium chloride solution of about physiological concentration.

FORCES OF WATER EXCHANGE RESIDING IN THE SKIN. Any exchange of water which is due to the force of osmotic pressure alone takes place at a rate proportional to the gradient of total concentration. This is merely a corollary of the definition of osmotic pressure. Over a short initial period of time, such as was used in the experiments, the internal osmotic pressure of the frog body is of a uniform value, or at most is changing slightly. Hence, whatever initial exchange is due to forces other than the one of osmotic pressure will probably not be proportional to the concentration of salt in the medium.

An analysis of the forces other than osmotic pressure which are concerned in the water exchange of the normal frog was carried out, based upon this principle (Adolph, 1927). The rate of movement of water which would be expected from osmotic pressure alone, in all concentrations of the medium, was inferred from the fact that in hypertonic solutions the rate of movement was found to be proportional to concentration. From this the rate of passage which would in hypotonic media result from the osmotic pressure alone was extrapolated.

A possible interpretation of the effect of salt concentration is to suppose that the skin when bathed by water on its outer surface is relatively impermeable to water, but that it becomes permeable or is "injured" in any

other medium. Such a statement merely names the phenomenon without analyzing it further.

In the experiments now reported the skinless frog and the pithed intact frog are found to behave like osmometers in which at all concentrations the initial movements of water are proportional to the concentration of the medium. The skinless frog and the pithed frog thus realize the ideal situation where the osmotic pressure of the medium, and hence the gradient of osmotic pressure, is the only variable in the initial rate of passage of water. This finding further justifies the previous analysis, and confirms

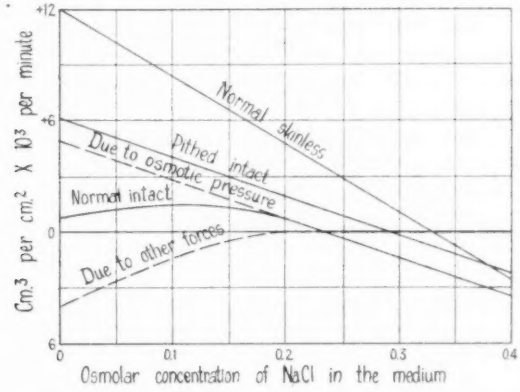

Fig. 8. Comparison of rates of initial intake of water in normal and in skinless frogs in relation to the concentration of sodium chloride in the medium. The curve for normal frogs takes into account the passage of that water through the skin which is simultaneously lost as urine; for pithed intact frogs and for normal skinless frogs this is disregarded, being negligibly small in comparison with the net rate of osmosis through the skin. The line "due to osmotic pressure" represents an extrapolation of the curve for normal intact frogs; and the line "due to other forces" depends upon it.

the conclusion that the forces other than osmotic pressure that are present in the intact frog are seated in the skin.

The relationships now to be considered are represented in figure 8. The results of the experiments with skinless frogs are represented by the two uppermost straight lines. The normal intact frog in sodium chloride is shown by a curved line; but this line is straight in the region of concentrations above 0.2 osmolar. This line is extrapolated to the lower concentrations, as above explained, for the purpose of representing what the force of osmotic pressure, due to sodium chloride, if effective alone, would do in moving water across the skin of an intact frog.

There is, in addition to the curves for skinless and for pithed frogs, very

direct evidence justifying the selection of this part of the normal frog's curve for extrapolation. This is the fact, found by Brunacci (1917) and Duval (1928), that when frogs are kept in sodium chloride solutions of 0.25 osmolar concentration or higher, their blood comes to have nearly the same freezing point as has the medium. Hence there are under such conditions no forces at work to make the concentrations unequal. Contrasted to this, in hypotonic media the blood was found never to come into concentrational equality with the environment.

If no forces but osmotic pressure controlled the passage of water through the skin, water would move into the normal frog when immersed in fresh water at 6 or 7 times the rate at which it actually does. To maintain itself in water balance, the frog would have to excrete twice its own bulk of urine per day instead of less than one-third its bulk as it actually does.

At all concentrations of the medium, the virtual rates of passage which are not due to osmotic pressure may be evaluated by subtracting the actual rates from the extrapolated values "due to osmotic pressure." This gives the lowest curve of figure 8. Initially these forces exist only in frogs that are in hypotonic concentrations of the medium, disappearing apparently immediately when frogs are transferred from water to any hypertonic concentration. In hypotonic concentrations they are modified slightly in value with the passage of time during which there takes place adaptation to the medium to which the individual is transferred, as has been evaluated previously.

In a similar manner the rates of chloride exchange may be analysed into those due to the osmotic pressure gradient of the salt medium and those due to other forces. From figure 5 it is found that the maximal or asymptotic rate of chloride intake by intact frogs in hypertonic media is 110 per cent of the body's content per osmolar gradient per hour. This corresponds to a chloride diffusion rate of 2.7×10^{-6} osmoles per osmolar gradient per cm.^{2} of frog's surface per minute. For skinless frogs the rates of chloride passage are found to be proportional throughout to the concentration of the medium, which suggests the extrapolation of the above rate for intact frogs down through the hypotonic range. At zero concentration it is thus found that a rate of -25 per cent of the body's content per hour, or 0.6×10^{-6} osmoles per osmolar per cm.^{2} per minute is due to other forces. This is a measure of the intensity of the forces which prevent the chloride from leaching out of the frog into its watery medium. When these rates are plotted against concentration of sodium chloride in the medium, the curves obtained form almost exactly a mirror image of figure 8. What relation these forces which inhibit the exosmosis of chloride bear to those forces which oppose the endosmosis of water, can be at present only surmised. They are probably very much smaller in magnitude.

The physical nature of these forces is not known, though certain superficial characteristics, such as their temperature coefficients (Adolph, 1930), are known. Analysis of the shape of the curve of rates "due to other forces," as drawn in figure 8, does not reveal anything conclusive; to state that these forces are inversely proportional to the logarithm of the concentration, leaves about as many possibilities concerning their nature as there were to begin with. Their reality has always been beyond reasonable doubt; for the existence of any organism in fresh water while water is exchanging through its skin, and its kidneys are not eliminating water at some huge rate, requires that the water attraction due to osmotic pressure is being opposed by other forces. If the nature of these forces could be discovered, the mechanism of regulating composition and volume would be understood.

The technical assistance of Mr. M. J. Nudo in carrying out the thousands of weighings represented in this and the following papers is gratefully acknowledged. Dr. W. O. Fenn rendered valuable criticisms in the planning and the interpretation of the experiments.

SUMMARY

1. The initial rates at which water was taken up or given off by frogs from which the skin had been removed were proportional to the concentration of sodium chloride in the medium.

2. When gelatin was added, at each concentration of sodium chloride, much less water was gained or more water was lost by the skinless frogs. The much greater rate of osmosis due to gelatin than to salt was accounted for by the rapid exchange of salt but not of gelatin between tissues and medium.

3. The influences of anesthesia, of pithing, and of the addition of gelatin to the medium were evaluated upon both skinless and intact frogs.

4. The skinless frog and the pithed intact frog are under certain conditions ideal osmometers. By comparing the rates of exchange which are due to osmotic pressure alone with the actual rates of exchange of normal intact frogs, those forces residing in the skin which ordinarily oppose the attraction of water by the osmotic pressure of the body fluids are evaluated.

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OSMOSIS INTO FROG SKIN, AND THE EFFECTS OF ISOLATION, OF ORIENTATION, AND OF THE BLOOD'S CIRCULATION

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When an intact frog is placed in a salt solution, it begins to take in water at a rate that is now known for each of various concentrations of sodium chloride. The initial part, at least, of this process of intake consists in the mere entrance of water into the skin. Does this superficial penetration of the skin proceed at rates similar to that of the skin isolated from the body? Are the rates of osmosis governed by the permeability of the tissue rather than by the rate of the blood's circulation or some other activity of the intact organism?

No problem could be simpler in conception; but obtaining and interpreting results requires attention to numerous factors. One of the most important factors is that the skin has a physiological orientation such that under otherwise similar conditions water passes through it much faster in one direction than in the other. This has long been known, and is attested by many experiments. If the rates of passage *through* the membranous tissue differ, it is at least to be suspected that the rates of entrance *into* the tissue will differ, depending upon which side of the tissue is exposed to the medium. In fact, Wertheimer (1925) studied the uptake of water from isotonic salt solutions, and reported that the uptake at the inner surface of isolated frog skin was several times more rapid than the uptake at the outer surface.

METHOD. Two series of experiments on the penetration of water into isolated skin were carried out, one in which the skin was exposed on both sides to the same solution, the other in which the skin was exposed on its outer surface only. In a third series of experiments the penetration of chloride into the skin from both sides was measured. In all series the media were solutions of sodium chloride made up in water that was distilled, stored, and used in pyrex glass.

Each frog (*Rana pipiens*) was pithed; a few experiments on skin taken from anesthetized frogs and from normal frogs showed no differences from skin taken from the pithed individuals. The entire skin was removed with the exception of the skin of the face, and was used entire. In stripping, the skin was turned inside out, and in most of the experiments to be reported

it remained inside out. In some experiments of series I the skin was turned back to its normal orientation; this position, however, made no difference in the rate of osmosis so long as all the cut edges of the membranous tissue were not tied off. Ordinarily edges occurred at the head, the cloaca, and at the tip of each of the four limbs. When these were tied off (series II) it was possible that the liquid not only entered the skin but passed through it and out of it on the other side, yet was measured as belonging within the tissue. In fact, however, only the very first minutes of osmosis were utilized in these experiments; in this period there was insufficient time for an appreciable amount of such passage completely through the skin to occur.

Each skin was used singly for an experiment. It was weighed immediately after being isolated, was placed in a solution, and was thereafter weighed at intervals. Each weighing was done in a weighing bottle, and during the determination the tissue drained itself of water; this water was then weighed with the bottle (to give the tare) after the skin had been lifted back to its solution. It is evident that exchange of fluid was thus not interrupted by the weighings. Each measurement of rate of osmosis consisted of five to twelve weighings At the beginning of the experiment the weighings were done at intervals of two minutes; later the intervals were increased gradatim, the last weighing being done at about one hour. For each measurement the weighings were plotted against the square root of the time after immersion of the tissue in the sodium chloride solution. In the majority, all the weighings fell along a straight line, within the limit of error in recognizing the time to which the weight belonged and the error of obtaining the correct net weight of the tissue. In a few experiments the weighings departed sufficiently from a straight line so that some choice existed in establishing the slope of that line. Instead of rejecting such experiments, care was exercised to have at least two experiments that were beyond dispute for every one whose interpretation was doubtful.

One guiding rule in obtaining the rate in doubtful experiments was that the fastest rate is usually considered the correct one, since most errors that might enter would cause delay in water uptake. In general, experiments where swelling was most rapid were the most regular in the individual weighings and continued in proportion to the square root of the time for the longest period. In higher concentrations, where swelling was slow, either positive or negative, it was usual to find two parts to the curve, composed of two straight lines with a sharp bend between them. The second slope was regularly taken in these instances, but due to the slow rate of osmosis prevailing the use of the first slope instead would have had little effect upon the final result in the concentration curves.

In most tissues the weight taken before immersion in the solution likewise fell upon the straight line extrapolated back to zero time. This shows

that fluid clinging to the exterior of the tissue in later weighings was negligible. In only one tissue was it found that this first weight did not fall on the straight line; this was for isolated skin exposed on its outside only (series II). It is correct to consider here that the skin was excessively desiccated during the minutes necessary for ligating the cut edges of it.

How is it possible for a tissue which is thin, such as a frog skin, to maintain a rate of osmosis that is proportional to the square root of the time for as long as ten minutes? In extreme experiments the skin doubled its weight in this period. Equations for estimating the saturation from the rate of osmosis such as those used by Andrews and Johnston (1924) and Hill (1928), cannot be applied to osmosis into frog skin, since the percentage of the theoretical saturation attained at any one moment differs for each concentration of the medium. The data themselves show that there is no time for initial swelling to occur at a greater rate than that indicated by the velocity as graphed, for when the later points are extrapolated back to zero time they strike the value of the tissue weight that was determined before immersion. It is safe to rely upon the exact relation to square root of time, and upon the fact that the rate of osmosis so calculated for isolated skin is nearly as great as the rate for a whole frog, a skinless frog, or a muscle, in which limiting thicknesses are excluded from consideration.

The chloride contents of isolated skin were determined (series III) by the procedure of Van Slyke (1923). In each determination the chloride found was calculated as the concentration belonging to the weight of the skin if it had not been subjected to shrinkage or swelling. In this way the effect of changes of water content in the skin was avoided; but actually the results are essentially alike with either basis of calculation. The concentrations as expressed are converted to osmolar units on the assumption that all the chloride was NaCl, in order to facilitate comparison with the concentrations of the medium.

For each experiment the frog was previously acclimatized to tap water at the temperature of the experiment. The skin as used constituted 12.2 per cent of the fresh weight of the skin plus the skinless body. There was no correlation between the percentage by weight of skin and the weight of frog, which means that the thickness of the skin was approximately as the linear dimension of the individual. The area of skin exposed was taken as equal to the area (series II) or to twice the area (series I) of the frog from which the skin was removed.

Series I. The rates of change of weight on the part of the skin were very rapid, as might have been expected from its large surface— mass ratio. Figure 1 shows that the initial changes of weight were proportional to the square root of the time. All the data are represented in figure 2. It will be seen that the individual determinations of the rate of skin swelling are about as divergent as those for intact or for skinless frogs. The average

values, at each concentration of NaCl in the medium, fall along a curve which is certainly a straight line. The point at which there was zero water

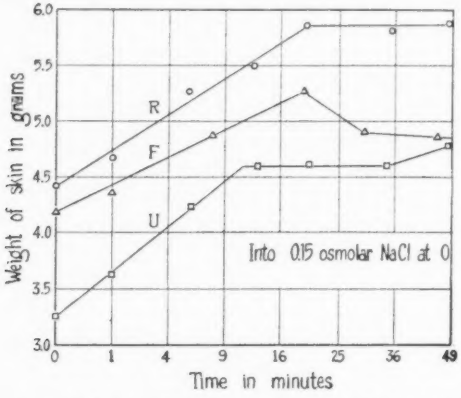

Fig. 1. Time courses of three experiments in which isolated skins were immersed in 0.15 osmolar solution of sodium chloride with both sides exposed. Time is plotted on a square root scale. The slopes of the curves for the first ten minutes are taken as the initial rates of osmosis.

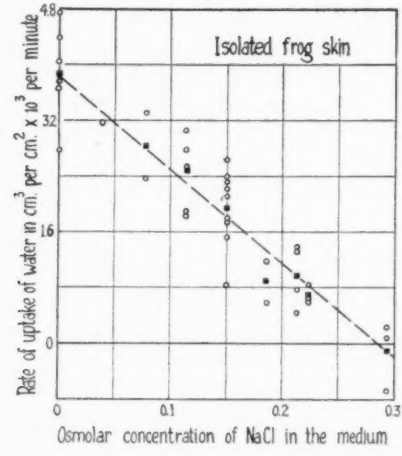

Fig. 2. Relation of initial rates of osmosis into isolated skins to the concentration of the medium. Each circle represents a single experiment; each black square the average for all the experiments at a single concentration. Temperature 19° to 27°C.

exchange occurs in a medium that is considerably above the molecular concentration of the blood.

The isolated skin, therefore, swells faster as the medium is more dilute. This is like the picture for skinless frogs and not like the picture for intact frogs. It could be said that the skinless body plus the separated skin behaves very differently from the body and the skin together in the intact individual.

It is worth while to point out that a different relationship is found between concentration of the medium and the amount of swelling if experiments are compared after a time longer than ten minutes. In figure 3 the

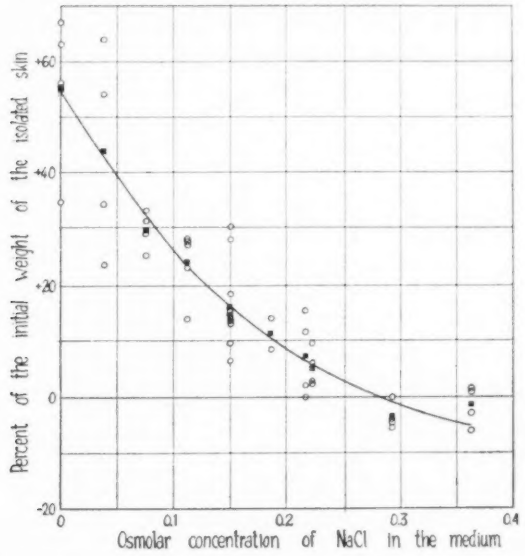

Fig. 3. Relation of increase of weight by osmosis into isolated skins to the concentration of the medium, if comparison is made by measuring the changes of weight after 30 minutes of immersion. Each circle represents a single experiment; each black square the average for all the experiments at a single concentration. Each skin was immersed in 100 cc. of solution at a temperature between 19° and 25°C.

correlation is made at 30 minutes; this graph includes some experiments that were not represented by weighings at intervals short enough for figure 2. Obviously the customary method of measuring the amounts of swelling in one hour or in 24 hours would have yielded little information of quantitative importance.

Series II. It was found by Wertheimer (1925) that frog skin in contact with M/8 NaCl swelled very differently if exposed on one side or on the other. From the neutral solution it took up water much more rapidly upon the inner surface than upon the outer surface; this could be reversed

by acid, the reversal presumably occurring at the isoelectric point of the skin's proteins. In the present experiments (series II) the attempt was always made to expose only the outer side by being sure that the skin formed a closed sack, tied at each extremity. At the end of every experiment the skin was tested for leaks, and the experiments on the few skins that were tied off incompletely were rejected.

Again the mean rates at each concentration fall along a straight line, as is shown in figure 4. While the slope of the straight line is slightly less than that of figure 2, there is certainly no curved portion to the concentration curve such as for the initial osmosis into normal intact frogs. The results on these skins are comparable to the initial rates of osmosis into pithed

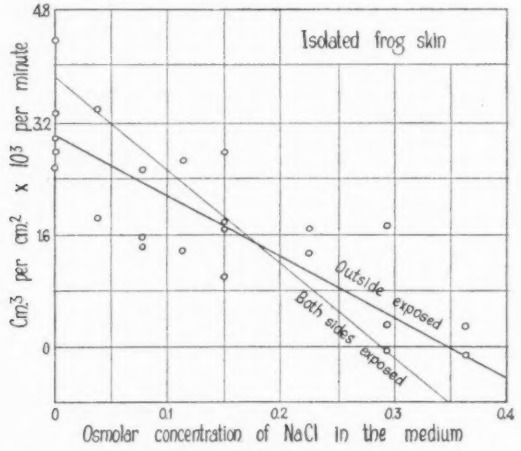

Fig. 4. Initial rates of osmosis into isolated skins that were exposed on the outer surface only to various concentrations of the medium. Each circle represents a single experiment with a single entire skin. Temperature 27°C.

intact frogs; such frogs have been shown to take up water at rates linearly correlated with the concentration of the medium. In this sort of experiment, therefore, the skin exposed on its outer surface imitates to some extent the intact body from which it came.

Series III. The finding that osmosis into isolated skin occurred at rates that are linearly related to the concentration of the medium, led to the study of the initial exchanges of chloride. Is the diffusion of solute directly correlated with the osmosis of solvent?

A series of determinations of chlorides was done after the skins had been immersed with both sides exposed to the medium for 30 minutes (fig. 5). In general the diffusion occurred as a linear function of the concentration of the medium. It is altogether likely that the curve, which is slightly

concave downward, would at 10 minutes or less be a straight line. In distilled water (zero concentration of the medium), an average of 85 per cent of the chloride contained in the skin leached out within 30 minutes. By this time the medium, which had about fifteen times the volume of the skin, had almost as high a concentration of this ion as did the tissue. Evidently most if not all of the chloride ion is in a state and in a location which allows it to leave the tissue.

The point at which no exchange of chloride occurs is in the region where 0.12 osmolar sodium chloride is contained in the medium. This is probably much lower than the total molecular concentration of the skin. At the

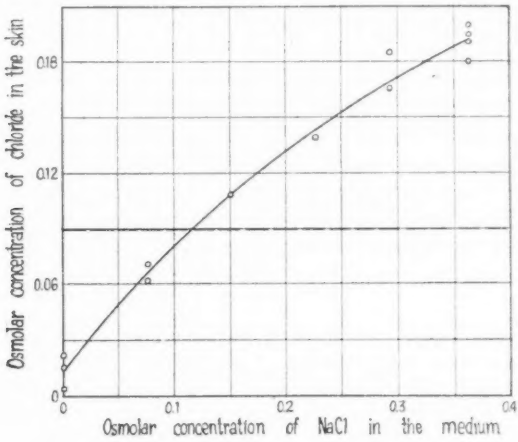

Fig. 5. Concentration of chloride found in isolated skins after 30 minutes of immersion in the concentration of medium indicated. Each circle represents the analysis of one entire skin. The horizontal dashed line represents the initial value found as the mean of 22 determinations upon unimmersed skins, which was 0.897 osmolar.

same time it is significantly higher than the initial chloride concentration of the skin. It is, then, not merely the gradient of chloride that is controlling the diffusion of chloride, though it might still be the gradient of chloride in some particular layer of the skin that is effective.

Whereas zero chloride exchange occurs in 0.12 osmolar solution, zero water exchange occurs in 0.28 osmolar solution. Between these concentrations both water and chloride are being taken up by the skin simultaneously. Outside these limiting concentrations, water and chloride are passing in opposite directions. Evidently the gradients and the forces concerned in the movement of the two substances cannot be altogether identical. The picture is no clearer if, as is sometimes done, the tissue is con-

sidered to have extracellular spaces that are physiologically distinct from the cellular masses.

THE INFLUENCE OF THE BLOOD'S CIRCULATION. Upon comparing the rates of osmosis into isolated frog skin under the influence of various concentrations of sodium chloride (fig. 4) with the rates of osmosis into pithed intact frogs under similar conditions (as described in the preceding paper), it was evident that the rates for isolated skin were at each concentration at least half of those for the skin on the pithed frog. The smallness of the difference seemed to indicate that neither isolation of the skin nor the absence of a circulation in the skin made much difference in the result, though it was possible, yet not probable, that these factors tended to neutralize one another. Experiments were therefore undertaken to attempt to measure the rôle of the circulation in osmosis.

The circulation of the frog may be studied qualitatively by observing the heart beat against the ventral body wall and by examining with a microscope the capillaries of the web or other superficial tissues. Flow in these indicates that at least some circulation is reaching the surfaces where materials are being exchanged with the environment. In a large series of experiments with anesthetized and pithed frogs, both intact and skinless, the circulation was thus watched. The supposition that the blood continued to flow for the duration of all experiments with salt solutions was vindicated.

Further than this, it was desirable to know quantitatively what circulatory modifications might occur. In the frog it is quite impossible to measure the volume flow of blood in the intact animal. Measurements were therefore made upon the cannulated perfused heart; these were performed in collaboration with Mr. Henry Brody, whose skill in double cannulation of the blood vessels is responsible for their success. The heart of the urethanized frog was left in situ, the ventral body wall and the pericardium were opened, and cannulae were inserted into a vein and into an aorta. The other aorta was tied off and Ringer's solution of varying strengths was pumped by the heart against a considerable and constant hydrostatic pressure. Solutions of strengths 0.22, 0.33 and 0.44 osmolar were furnished under a uniform low head of pressure, and the arterial output per minute was measured practically continuously.

There was no regular difference of heart output with diverse concentrations of the perfusing fluid. It was found that every change of concentration, whether from high to low or from low to high, induced a burst of output which after some minutes subsided. After the subsidence the rates averaged slightly lower with the more concentrated solutions in two frogs, and considerably higher with the more concentrated solutions in one frog. The same response was repeated a number of times in each individual. It was found that only occasionally did the rate of heart beat change in

parallel to the volume output; it would have been highly erroneous to judge of the circulatory volume flow from counts of the pulse rate.

Concentrations of perfusing fluid up to 0.44 osmolar did not, then, markedly change the rate of the circulation. Only at about 0.50 osmolar was the circulation slowed and eventually stopped. Since the conditions to which frogs were subjected in the course of the osmosis experiments produced only gradual changes of concentration in their mixed blood, it may be concluded that within the short time of 30 minutes, and usually only 10 minutes, during which the experiments lasted, there was no definitive change in the rate of the circulation.

Under other conditions it appears that the effect of the increased concentration of the medium upon the heart beat must be the limiting condition for

TABLE 1

Rates of initial osmosis at 27°C. into intact frogs with the circulation stopped by injections of paraffin oil, compared with the rates into normal intact individuals

DESIGNATION OF FROG	CONDITION OF CIRCULATION	CONCENTRATION OF NaCl, OSMOLAR	RATE OF OSMOSIS, $\frac{cm^3 \times 10^{-3}}{cm^2 \times \sqrt{MIN.}}$
b	Normal	0	+1.9
a	Stopped	0	+2.9
i	Normal	0.150	+2.1
q	Stopped	0.150	+4.4
j	Normal	0.363	−2.8
l	Stopped	0.363	−1.8
k	Normal	0.363	−3.2
p	Stopped	0.363	−3.4

the survival of the individual. Intact frogs usually die in two or three hours in any concentration of solutes above 0.4 osmolar. Skinless frogs die in about half an hour in any concentration above 0.4 osmolar. From the data on chloride penetration it can be realized that the changes of concentration in the blood are then probably sufficient to accomplish death by stopping the heart, for in skinless frogs the average chloride concentration of the whole body increased by 0.08 osmolar in half an hour.

Attempts were made to stop the circulation without causing other primary injury, and thereafter to study the rate of osmosis into these intact frogs without a circulation. The method was to insert a needle attached to a syringe through the ventral body wall until the heart was pierced, as evidenced by withdrawing blood through the needle. Paraffin oil was then

injected from the syringe. Although blood could always be withdrawn, the circulation was stopped as the result of injecting the oil in only one out of every eight frogs so treated. This is taken to indicate that in the other cases the oil did not get into the heart. Each of the frogs in which the injection was successful were transferred from tap water to a solution of sodium chloride just as soon as the circulation ceased and the frog became limp. With each such frog a normal frog was put into the same solution, and the changes of weight were followed on both. The results are given in table 1. There were no significant differences in the rates of osmosis into or out of the body with the circulation stopped as compared to the normal rates. So far as the experiments indicate, the circulation appears to be unnecessary for the exchange of fluid to take place at its normal rate through the skin. Such a conclusion might account for the oft-repeated observation, recently mentioned by Brugsch (1928), that a "killed" frog takes up water about as fast as a normal one whose urine is being retained.

A circumstance that may or may not be significant of the rôle of the circulation is that, since the amount of water entering the body of the frog is proportional to the square root of the time rather than directly to the time, the conditions are not those for osmosis in a steady state. It is as if the water was carried away from the skin by the blood so slowly that it actually accumulated in the tissues near the body surface, and did not wait for its entrance to be retarded until accumulation was proportional throughout the body. But it is possible that other factors than accumulation are responsible for the correlation of amounts of osmosis with the square root of the time.

Absorption of water by skin. It must be borne in mind that the rates of initial osmosis that have been measured are the resultants not only of the relative attractions for water exerted by the tissues and by the media, but also of the very imperfect semi-permeability of the tissues to non-colloidal solutes. Differences or similarities in rates of osmosis may possibly be due to compensation of one of these two factors by the other. A more rapid leaching out of solutes may counteract a greater initial osmotic pressure tending to draw water inward.

When exposed to solutions of sodium chloride, isolated skin initially takes up water at rates in linear relation to the concentrations of the solutions. In this respect it resembles osmotically a pithed intact frog or a skinless frog. There is little or no significant difference between the rates of entrance of water into isolated skin when exposed on both sides and when exposed only on its outer surface. Differences of the magnitude reported by Wertheimer (1925) on skin similarly isolated were not found. Osmosis into the outer surface of the isolated skin as used in these experiments therefore does not resemble osmosis into a normal intact frog.

The rate of uptake of water per unit of external area exposed is at least

half as rapid in the isolated skin as in the pithed intact frog. Evidently when the intact frog is transferred from water to any solution of sodium chloride, the initial exchanges of water depend primarily upon the conditions of osmosis between the outer layers of the skin and the medium. The circulation and the internal tissue may influence subsequent exchanges and equilibria, but initially the skin takes its own course in carrying out the exchanges. It is these processes of initial osmosis into a solid body that are the limiting factors in the exchanges of water that have been investigated.

SUMMARY

1. Freshly isolated frog skin initially gains or loses water in proportion to the square root of the time elapsed after its immersion. In solutions of sodium chloride to which it is fully exposed, the gain or loss is linearly related to the concentration of the salt in the solution.

2. If exposed only on its outer surface, the skin taken from pithed frogs likewise gains or loses in linear relation to the concentration of sodium chloride in the medium, at rates at least half of those found in the pithed intact frog. This fact refers the initial exchanges of water by the intact frog to the skin itself.

3. Chlorides are exchanged by isolated skin at rapid rates which are in linear relation to the concentration of sodium chloride in the medium.

4. The rate of the circulation bore no measurable relation to the concentration of the fluid being transported by it. In a few experiments in which the circulation was stopped altogether, the rates of osmosis into or out of intact frogs were the same as for normal intact frogs.

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THE INITIAL RATES OF SWELLING OF ISOLATED MUSCLE AND THEIR RELATION TO THE OSMOTIC EXCHANGES WITHIN THE FROG

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In attempting to gain an insight into the distribution of water throughout the tissues of the frog's body, it is indispensable to study the type of tissue that bulks largest in the body. Isolated muscle has received considerable attention in past studies of swelling; but when the data so reported were translated into terms of rates of osmosis, it was found that they could not be compared with the data that have now been obtained upon other tissues, chiefly because amounts of swelling had been measured only after long periods of time. These studies had been made primarily in the interest of some theory of contraction or of narcosis, and often an equilibrium between muscle and medium was sought. The object of the measurements now to be reported was to compare, in the first few minutes of immersion in various solutions of sodium chloride and gelatin, the rates of osmosis into whole isolated muscle with the rates of osmosis into the skinless frog, in which muscles are the chief tissues exposed to the medium. From the data now at hand osmotic constants are calculated for the several tissues of the frog; and the relation between the rates of osmosis into isolated frog tissues and the partition of water within the frog's body is defined.

METHOD. Two kinds of muscles were prepared from freshly pithed individuals of *Rana pipiens* that were previously acclimatized to tap water at the temperature of the experiment. One was the gastrocnemius, severed at its tendon on the distal end, and cut off at the surface of the femur on its proximal end. The other was the entire upper leg, obtained by cutting through both bone and muscles at the knee joint and at the hip joint. The first preparation gave a single muscle with practically intact surfaces; the second a group of muscles, supported by a core of bone, with intact surfaces on all longitudinal borders and cut surfaces on all transverse borders. A third kind of muscle, used in a couple of determinations, was cut from the thin ventral body wall. Exchanges of water were measured as changes of weight over very short periods of time; the technique of weighing was that already described for frog skin.

It was necessary to relate the osmosis that occurred with the amount of surface of muscle exposed to the medium. Each preparation was measured in three axes; but the differences among all gastrocnemii and among all upper legs were so small that it was sufficiently accurate to take all of each kind as having the same surface area. In fact, no better agreement among experiments was obtained when the areas were taken to vary as the two-thirds power of the weights.

All gastrocnemii were considered to be prolate ellipsoids of revolution having axes of 2.40 cm. and 0.64 cm.; and therefore a surface of 3.8 sq. cm. All upper leg preparations were considered to be prolate ellipsoids of revolution having axes of 3.30 cm. and 1.30 cm.; and therefore a surface of 11.0

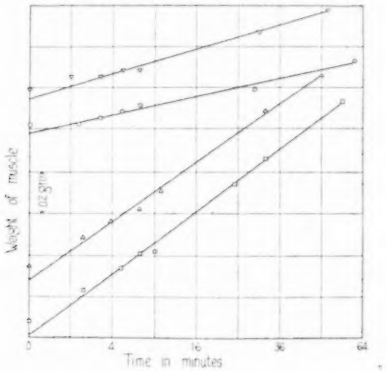

Fig. 1. Changes of weight in four muscles taken from one frog and immersed in 0.038 osmolar sodium chloride at zero time. The upper two curves are for the gastrocnemii, the lower two curves for the upper-leg preparations. Temperature 27°C.

sq. cm. The ventral body wall pieces were measured as rectangular parallelepipeds.

SODIUM CHLORIDE SOLUTIONS. In solutions of all concentrations the changes of weight of the muscle preparations were found to be initially proportional to the square root of the time elapsed after immersion. The graphical comparison of the five to twelve weighings made within an hour thus gave a slope based upon this number of points, as is shown in figure 1. In the lower concentrations of the medium the initial linear relation held for about one hour, but in the higher concentrations it was necessary to reject the first ten minutes of the experiment and to take the slope thereafter, just as was done for skinless frogs. It requires one hour for muscles in distilled water to become turgid to the point of rigidity; in other solutions they do not become rigid.

The proportionality of osmosis to surface area is shown by comparing

the rates of osmosis of the two upper leg preparations and the two gastrocnemii that are included in figure 1.

The initial rates of osmosis when muscles were plunged into solutions of pure sodium chloride are plotted in figure 2. It will be observed that the data for the two kinds of muscle preparations at any one concentration fall within identical ranges.

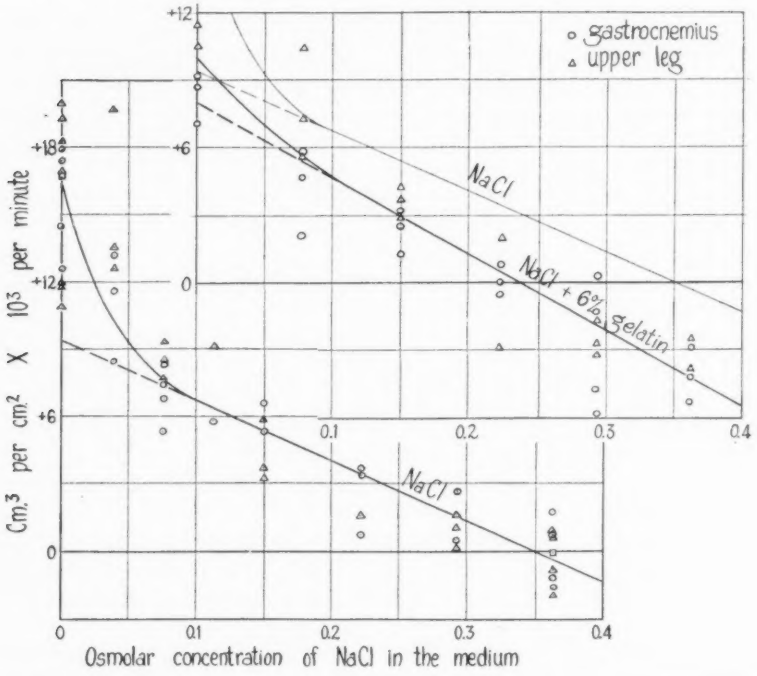

Fig. 2. Initial rates of osmosis into isolated frog muscles at various concentrations of sodium chloride in the medium. Each circle represents one gastrocnemius muscle, each triangle one upper-leg preparation, and each square a body-wall piece. Temperatures ranged from 19° to 27°C. Lower graph, sodium chloride alone; upper graph, sodium chloride containing 6 per cent of gelatin. The upper thin line repeats the lower graph.

The mean values at each concentration fall along a line that is sensibly straight between 0.075 and 0.365 osmolar. Between zero concentration and 0.075 osmolar there is a marked increase above the values extrapolated from the straight line. This fact is attested by every one of the eighteen points obtained in these lower concentrations. It is also attested by some measurements of Buglia (1909) on gastrocnemii of Rana temporaria,

which are the only previous data that compared amounts of swelling in various sodium chloride concentrations at so short an interval after immersion as 15 minutes. At the interval of one hour, the data of Korösy (1914) exhibit an exaggeratedly similar arrangement.

The shape of the curve in figure 2 is different from that for any tissue or organism that has been studied thus far. Its upward trend contrasts with the downward trend in the same low concentrations on the part of the intact frog. But while the skin of the intact frog is usually in contact with these low concentrations, the muscles of the intact frog probably never come in contact with any fluid so dilute as 0.075 osmolar.

Sodium chloride + gelatin solutions. In another series of experiments, 6 per cent of gelatin was added to the various concentrations of sodium chloride. The average points (fig. 2) again fell approximately along a straight line except at very low concentrations. This line has a somewhat greater slope than the line for sodium chloride alone. In the higher concentrations of salt the gelatin caused water to leave the muscles instead of entering them. Thus it is seen that isolated muscles are influenced like skinless frogs by the presence of gelatin in the medium.

That colloidal substances are particularly effective in attracting water away from frog muscles was noticed by Overton (1902). In certain of his experiments 1 per cent of dextrin, when added to a concentration of sodium chloride which by itself produced no exchange of water, drew water from gastrocnemii. In a few experiments Beutner (1913) added 2 per cent of gelatin to the sodium chloride solutions in which gastrocnemii were immersed. He did not consider that the differences in changes of weight were significantly diverse whether the sodium chloride medium contained gelatin or not.

Results were reported by Schade (1923) upon the swelling of isolated human connective tissues that were immersed in blood serum containing diverse concentrations of albumin. Though the amounts of swelling were compared only after 24 hours had elapsed, undoubtedly the same colloidal properties rendered the protein more effective than salt in attracting water from the tissue. In contrast to gelatin and similar substances which do not penetrate the tissues, sodium and chloride penetrate them very rapidly and so are relatively ineffective in exerting osmotic pressure.

Higher concentrations of gelatin were tested upon muscles, with 0.235 osmolar sodium chloride in the medium. The results, given in figure 3, show that in these concentrations of gelatin more and more water was withdrawn from the muscles. But the additional concentrations were not proportionally effective, just as was the case with skinless frogs.

Comparison with skinless frog. There is one important diversity between the skinless frog and the isolated muscle. This is the fact that the

straight-line relationship between initial osmosis and concentration of sodium chloride holds throughout in the skinless frog, while it does not hold at concentrations below 0.075 osmolar in the isolated muscle. In this one range the data constitute an objection to considering the skinless frog as a mere exposure of muscles.

In higher concentrations the slope of the line for rates of osmosis per unit of surface is somewhat less for isolated muscle than for skinless frog. Water is initially neither gained nor lost by the muscle and by the skinless frog at about the same concentration (0.33 to 0.35 osmolar). There is nothing to indicate what factors are responsible for these slight differences

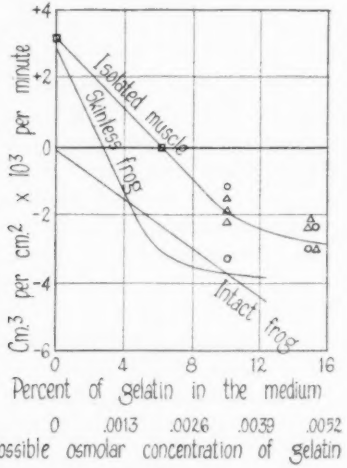

Fig. 3. Initial rates of osmosis into frog muscles at various concentrations of gelatin in the medium containing 0.235 osmolar sodium chloride. Each circle represents one gastrocnemius, each triangle one upper-leg preparation, and each square is an average result taken from figure 2. Temperature 27°C. The two lower curves repeat the average results for the preparations named.

between muscle and skinless frog, except the obvious fact that tissues other than muscle are exposed to osmotic processes in the latter preparation. The close similarity of isolated muscles to skinless frogs strongly confirms the conclusion that the circulation has no importance in the initial exchanges of water.

Relation of initial osmosis to equilibrium. An experiment repeated by many investigators was to find the concentration of sodium chloride in which a frog muscle would not swell. The concentration found had various values, ranging from 0.20 osmolar (Loeb, 1898) to 0.29 osmolar (Leduc, 1905). In figure 2 the value is 0.35 osmolar. This higher value

depends upon using the rates of osmosis as here evaluated, in which the first ten minutes of water exchange are disregarded, instead of using the interval of an hour or more from the weighing just before immersion, for estimating the exchanges of water.

The concentration of sodium chloride required for zero swelling is very different when gelatin is present. With 6 per cent of gelatin in the medium, only 0.235 osmolar sodium chloride is required to prevent the weight of the muscle from increasing.

If muscles are left in Ringer's solutions of various concentrations for many hours, characteristic changes are produced in the osmotic pressure of the medium, which Hill (1930) interpreted as being proportional to the changes of vapor tension of the muscle. There is no evidence that either vapor tension or change in vapor tension is ever equal inside and outside the muscle. Very different exchanges would be obtained, for instance, if 6 per cent of gelatin were present in the Ringer's solutions, yet this concentration of liquid gelatin is said to have an effect upon the vapor tension of its solution equal to that of only 0.001 molar sodium chloride. Other substances beside chloride and water are interchanged with the medium by muscle to varying extents, and they in turn attract water in proportion to their osmotic pressures as exerted across membranes that are changing in permeability during the progress of losing their vital state.

If the leg muscles of normal *Rana pipiens* are boiled, the juice squeezed out, and the freezing point of this juice determined, its molecular concentration is found to be 0.325 osmolar (Adolph, 1927). The intact muscle's proteins exert additional osmotic pressure, which is probably not as great as that exerted by 6 per cent of gelatin, for zero water exchange at 0.325 osmolar sodium chloride would be obtained with only one per cent of gelatin in the medium. Possibly other forces of unknown magnitude are being exerted in addition to that of osmotic pressure. It is plain that a solution cannot be said to be "isotonic" with muscle merely on the evidence that the muscle does not change its weight when immersed in it, for solute may be rapidly penetrating into or out of the tissue simultaneously with the equilibration of water.

Attempts have been made to compare whole muscles with chopped muscles with respect to their swelling (Winterstein, 1916). But the rates of osmosis cannot be compared, since the surface of chopped muscle cannot be measured. Korösy (1914) compared whole muscles with muscles whose exterior surfaces were peeled away. It is likely that the faster rate of swelling as measured in percentage gain of weight was in the latter also due to the increase of surface-mass ratio.

Comparison of rates of osmosis. In this and the foregoing papers all initial rates of passage of water into or out of frogs or their tissues

have been measured as gm. or cm.^{3} of water entering a cm.^{2} of surface in (the square root of) one minute. The rates found in individual experiments were plotted against the concentration of sodium chloride in the medium in which the experiments were done. Average curves were thus obtained, and these curves are brought together in figure 4 for all the cases where pure sodium chloride solutions constituted the medium.

Similar curves were obtained in which the sodium chloride solutions contained 6 per cent of gelatin. All these average curves are reproduced in figure 5.

Finally, curves were obtained in which the solutions were varied with respect to the concentration of gelatin, always containing in addition 0.235

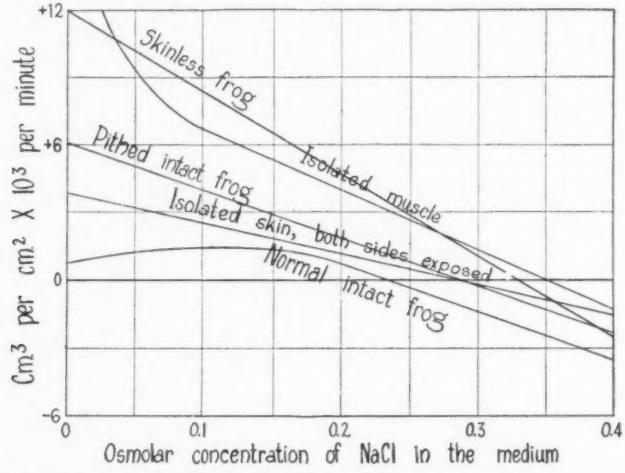

Fig. 4. Initial rates of osmosis plotted against the concentration of pure sodium chloride in the medium. Each curve is the average result for the preparation named.

osmolar sodium chloride. These average curves are compared in figure 3. It is by no means certain that the osmotic pressure exerted by the gelatin is proportional to the number of grams of gelatin that were made up in 100 cc. of solution.

The curves of any one of the three charts are sufficiently far apart so that it can be safely concluded that no two of them are identical. The diversities are particularly important in those cases where the slopes differ, for these diversities appear to be chiefly due to unlike permeabilities of the tissues to sodium chloride. But this is no explanation of the diversities shown in figure 3.

Osmotic constants. It has been found for each of the tissues studied that the amount of water entering it initially is proportional to the square

root of the time elapsed since immersion of the tissue in the medium. In bringing together the individual experiments, it was found that the volumes of water entering the tissue per unit of time were proportional to the surface areas of tissue exposed. Finally, in plotting the results against the concentration of the medium, at least a part of each curve turned out to be a straight line. These three empirical correlations make possible the rational calculation of a constant for the rate of osmosis.

The osmosis of solvent, albeit the process has a distinctive name, depends upon the same factors as the diffusion of solute. Where the material accumulates in each layer, and its egress is inhibited by confinement, the case is like the one first studied by Stefan (1878) of the entrance of a

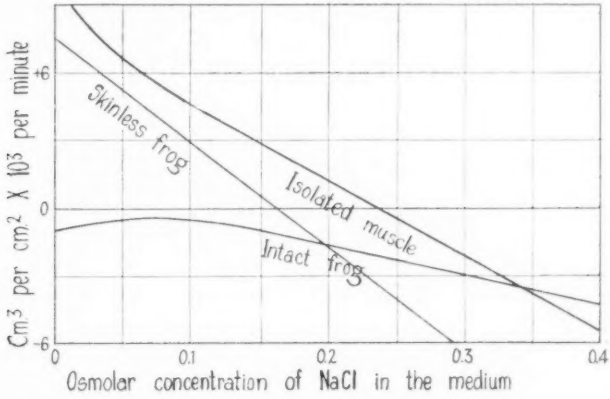

Fig. 5. Initial rates of osmosis plotted against the concentration of sodium chloride in the medium containing also 6 per cent of gelatin. Each curve is the average result for the preparation named.

gas into a test-tube. Eggleton and Hill (1928) used a similar formula for passage of material into such a "semi-infinite" space, which is that

$$S = 2\,c\,q\,\sqrt{\frac{k\,t}{\pi}}$$

In this equation S is the amount of substance passed, c is the gradient of concentration of the substance, q is the area of surface across which passage occurs, k is the coefficient, and t is the time elapsed since passage of the substance began. It should be noted that into this equation the thickness of the space does not enter, except as a limitation upon the duration of the experiment.

Such an equation contains all the factors in their correct dimensions as found for osmosis into frogs and their isolated tissues by the empirical

correlations mentioned above; S is proportional to each of $\sqrt{t}$, q, and c. For the sake of comparison with diffusion coefficients, therefore, the factor $2/\sqrt{\pi}$ is retained, and the factors in the equation are put into units of centimeters and minutes. For water it is necessary to consider that one osmolar of osmotic pressure is equal to a gradient of concentration of

TABLE 1

Osmotic constants for water in frog tissues at 27°C., calculated on the basis of the influence of the concentration of sodium chloride in the medium

TISSUE	KIND	NUMBER OF INDIVIDUALS PER DETERMINATIONS	NUMBER OF DETERMINATIONS	MEAN WEIGHT OF WHOLE FROG	MEAN WEIGHT OF TISSUE	MEAN SURFACE OF TISSUE	HALF-THICKNESS OF TISSUE (WEIGHT/SURFACE)	OSMOTIC CONTENT k	CONCENTRATION OF ZERO WATER EXCHANGE p
In NaCl solutions									
				gm.	*gm.*	*cm.²*	*cm.*	*cm.²/min.*	*osmolar*
Intact	Normal	3	49	37	37	86	0.43	1.05	0.23
	Anesthetized	3	22	45	45	97	0.46	—	0.30
	Pithed	1	25	30	29	74	0.39	0.78	0.29
Skinless	Normal	1	40	36	30	84	0.36	3.22	0.33
	Anesthetized	3	21	33	28	79	0.35	3.47	0.31
	Pithed	1	23	34	29	80	0.36	3.46	0.32
Skin	Both sides	1	45	36	4.6	169	0.027	0.43	0.29
	Outside	1	23	40	4.0	90	0.044	0.18	0.35
Muscle	Gastrocnemius	1	23	34	0.8	3.8	0.21	1.74	0.35
	Upper leg	1	25	34	3.6	11.0	0.33	1.50	0.37
In NaCl + 6 per cent gelatin									
Intact	Normal	3	31	49	49	103	0.47	0.42	(0.08)
Skinless	Normal	1	21	37	31	87	0.36	5.23	0.16
Muscle	Gastrocnemius	1	18	45	0.8	3.8	0.21	2.18	0.22
	Upper leg	1	16	45	3.6	11.0	0.33	3.04	0.26

18 grams of water per liter. The relationship $c = 0.018\ (m_1 - m_2)$ is therefore used, m being the osmolar concentration of the medium. The values of $S/c\sqrt{t}$ are read off from the slopes of the straight portions of the curves in figures 3, 4, and 5. The constant k is therefore an expression for the square of the slope of each curve, while the concentration of zero water exchange, represented by p, locates each curve on the ordinates

and abscissae. The ordinates along the curves, termed w, may then be located on all straight portions of the curves by the relation

$$w = \frac{S}{q\sqrt{t}} = 2 \times 0.018 \sqrt{\frac{k}{\pi}} (p - m) = 0.0203 \sqrt{k} (p - m)$$

The constants k and p are the basis of comparison among the truly quantitative "plasmolysis" experiments reported.

First the data may be compared in relation to the concentration of pure sodium chloride in the medium; the values of k derived from these data, together with other relevant facts, are listed in table 1. All the values of k for frog tissues, calculated on the basis of the concentration of this salt in the medium, are seen to lie between 0.2 and 5.2 cm.^{2} per minute. Some of these results were previously reported in abstract (Adolph, 1930).

TABLE 2

Osmotic constants for water in frog tissues at 27°C., calculated on the basis of the gradient of gelatin in the medium

Sodium chloride of 0.235 osmolar was always present with the gelatin. One per cent of gelatin is taken to be 0.000,32 osmolar.

TISSUE	OSMOTIC CONSTANT k	CONCENTRATION OF GELATIN FOR ZERO WATER EXCHANGE p
	cm.^{2}/min.	*osmolar*
Intact frog	3,100	0
Skinless frog	27,500	0.0008
Isolated muscle	6,200	0.0019

But the gradient of sodium chloride has already been shown not to represent the full effect of the corresponding osmotic pressure. The data for osmosis in relation to the concentration of gelatin, however, seem to represent the true effect of the initial difference in the concentration of water. From figure 3 these values of k are calculated, and are listed in table 2; they are seen to lie between 3,100 and 27,500 cm.^{2} per minute. They contain a small effect due to the diffusion of salts at the same time as the osmosis of water.

It is interesting to compare the values of k for osmosis into frog tissues with certain values of the constant that can be calculated by the same formula from the data of other investigators. The calculations are justified by the fact that in each instance (except for erythrocytes) S was shown empirically to be proportional to c and to $\sqrt{t}$. The values found are shown in table 3. The data on the frog blood-capillary were obtained by Landis (1927) in comparing the rate of filtration of water between blood and mesentery tissue with the hydrostatic pressure of the blood in the capillary.

While osmosis from capillaries ordinarily occurs in a steady state, the conditions of these measurements were such that the steady state was destroyed, indicated also by the fact that the rate of osmosis was proportional to the square root of the time and not directly to the time. Hydrostatic pressure thus yielded values of the osmotic constant that are intermediate between those yielded on other tissues by osmotic pressure of gelatin and by osmotic pressure of salt. The osmotic pressure exerted by salt furnished the gradient in the other determinations listed, namely, on sea-urchin eggs and human erythrocytes, and the constants in these microscopic tissues are much smaller than for salt osmotic pressure in frog

TABLE 3

Some osmotic and diffusion constants as calculated by the formula $k = \frac{\pi S^2}{4tc^2q^2}$

SUBSTANCE	TISSUE	EXPOSED TO	k	INVESTIGATOR
Water	Sea urchin egg (unfertilized)	Diluted sea water	0.000,46	Lillie (1916)
Water	Sea urchin egg (fertilized)	Diluted sea water	0.001,33	Lillie
Water	Human erythrocyte	NaCl solutions	0.000,6	Jacobs (1927)
Water	Frog blood-capillary (oxygenated)	Hydrostasis	24.	Landis (1927)
Water	Frog blood-capillary (asphyxiated)	Hydrostasis	580.	Landis
Water	Intact frog	Gelatin solutions	3,100.	Adolph
Water	Frog muscle	Gelatin solutions	6,200.	Adolph
Water	Intact frog (in hypertonic)	NaCl solutions	1.05	Adolph
Water	Frog muscle	NaCl solutions	1.74	Adolph
Water	Frog skin (both sides exposed)	NaCl solutions	0.43	Adolph
Lactate	Frog muscle	Na lactate	0.000,02	Eggleton (1928)
Phosphate	Frog muscle	Na phosphate	0.000,005	Stella (1928)
Chloride	Frog skin	NaCl	0.000,007	Adolph

tissues. This gives rise to the probability that the ova and erythrocytes lose their solutes as readily as the tissues of frogs do. There is no special reason for expecting all osmotic constants to be alike in value, except where nothing else besides water is diffusing, and in the presence of uniform partitions.

When the amounts of water that have entered any one kind of frog tissue at a definite interval of time are compared with the amounts that might be supposed to enter by the time theoretical "saturation" or isotonicity is reached, it is found that the proportion of one to the other is not constant for different media. In other words, k cannot be calculated from the

velocity with which saturation is approached, as it can for the entrance of water into sheets of rubber (Andrews and Johnston, 1924) and for the entrance of solutes into muscle (Hill, 1928). The essential differences may lie in the facts that water remains the phase of largest bulk in the tissues, and that solutes are progressively being exchanged with the medium.

DIFFUSION COEFFICIENTS. Using the same formula for passage of substance into and out of a solid body, diffusion coefficients may be calculated for the passage of solutes into frogs and their tissues. Table 4 shows the values derived from the present measurements of chloride exchanges. The values of k are less representative of the curves obtained, because for some of the tissues the slopes of the curves for diffusion rate

TABLE 4

Diffusion coefficients for chloride in frog tissue

TISSUE	NUMBER OF DETERMINATIONS	MEAN WEIGHT OF WHOLE FROG	MEAN WEIGHT OF TISSUE	MEAN SURFACE OF TISSUE	MEAN HALF THICKNESS OF TISSUE	DIFFUSION CONSTANT k	CONCENTRATION OF ZERO CHLORIDE EXCHANGE p
		gm.	*gm.*	*cm.²*	*cm.*	$\frac{cm.^3}{10^6 \times min.}$	*osmolar*
In NaCl solutions:							
Intact frog	17	36	36	84	0.43	6	0.00
Skinless frog	11	34	28	81	0.35	270	0.06
Skin, both sides	13	35	4.4	165	0.027	7	0.12
In NaCl + 6 per cent gelatin:							
Intact frog	2	32	32	78	0.41	21	0.00
Skinless frog	10	34	28	81	0.35	260	0.09

plotted against concentration depend upon extrapolated values. The coefficients are very large for skinless frogs, and are low for the penetration of skin. In the case of chloride, therefore, it is the skin that limits the exchanges of chloride between the body and the medium. Relatively the skin appears to retard the exchange of chloride much more than the exchange of water. Though by comparison to the skinless frog the coefficient for isolated skin is low, yet it must be realized that in 0.9 osmolar solution of sodium chloride the skin would double its chloride content in one minute, or in pure water would lose one-sixth of its chloride content in one minute.

Determinations of the diffusion of lactate and of phosphate into frog muscle have been reported by Eggleton (1928) and Stella (1928), and their mean values are shown in table 3. In those determinations the exchange

was proven to be proportional to the square root of the time, and was over most of the range of concentrations proportional to the concentration of the medium. It is interesting that the coefficients for lactate and phosphate diffusing into muscle are of the same magnitude as the coefficient for chloride diffusing into skin, whether the skin be on or off the body. For the skinless body the coefficient is much higher.

The coefficients for solute diffusion are of an entirely different order of magnitude from the osmotic constants for water osmosis. This might mean that the solvent or medium belongs in a class by itself. The difference suggests more strongly, however, some relationship to the great amounts of water already present in the tissue as compared with the small amounts of solutes therein. Or, it is possible that the constant has not the same meaning for solute and for solvent.

INTERRELATIONSHIPS OF TISSUES. The results thus far obtained in the study of the rates of initial osmosis into tissues of the frog may best be integrated by considering the interrelationships of these tissues in the functioning organism. Ordinarily the blood system serves to distribute water among all tissues. But the relative avidities of the tissues for water conceivably are the same, whatever the path of distribution of the water. The initial rates of osmosis are, under certain limitations, measures of the forces that move and that hold water. At equilibrium the forces are neutralized and so little can be found out about them; the attempt is therefore made to use the initial rates of osmosis, or the osmotic constants calculated from them, as the basis for predicting the dynamic equilibria of water among the tissues of the body, and between the body and the medium.

In the actual experiments each kind of tissue studied had its rates of osmosis measured when it was immersed in solutions of sodium chloride and gelatin. It may be inferred that the specific rate of osmosis between two tissues, if one of them could be immersed in the other, would be equal to the algebraic sum of the two rates as measured when each tissue is separately immersed in a common medium. In the body the various tissues are not immersed in one another, but actually all are adjacent to a common tissue, the blood, and to a much less extent to its filtrate the lymph. Their exchanges with this common medium constitute the partition of water in the organism. The exchanges of water do not occur between the morphological surfaces of each tissue and the blood, but across the walls of the blood capillaries. Whether the capillary endothelium has any influence upon the rates of exchange, or constitutes a limiting factor, is not known.

The concentration at which no water exchange occurs, that is, the value of p for the tissues, that would prevail when blood is the medium, has not

been measured. Landis found that a hydrostatic pressure of 11.5 cm. of water was required to keep water from passing from mesentery into blood of the frog; a pressure that theoretically would be compensated by a difference of effective concentration of 0.0005 osmolar, which is equivalent to 1.5 per cent of gelatin. But since the elastic pressures of the capillary walls and the mesenteric tissue were not evaluated, this value merely confirms the assumption that the tissues and the blood are ordinarily in approximate dynamic equilibrium with respect to water.

When a muscle or other tissue is excised from the body of a normal frog, this muscle is out of static equilibrium with all other tissues only to the extent of its small continuous requirement of water. If, then, excision involved no physiological change in the tissue, it would be expected that all the tissues would show approximately zero rates of osmosis at some one concentration of the medium. Unfortunately, only two of the preparations studied are normally wholly bathed by blood and lymph. The curves for these two, muscle and skinless frog, cross one another near the zero osmosis line in figure 4. Complete isotonicity of muscle and of skinless frog, that is, no initial exchange of water between them, can be established only in a medium that has the complete composition of blood plasma; but it may be approximated in a blood whose value of p is about 0.27 osmolar pure sodium chloride, as can be seen in figure 4.

If it be assumed that the data recapitulated in figures 4 and 5 are not influenced by the excision of the tissues, then the equation containing the specific osmotic constant may be used to calculate the rates of osmosis, or the net forces that attract water, between any two tissues t and v. When in contact with blood whose value of p is the osmolar concentration m, the initial water exchange between them will equal

$$w_t - w_v = 0.0203\,[(p_t - m)\sqrt{k_t} - (p_v - m)\sqrt{k_v}]$$

The forces will be almost the same at equilibrium as initially, so long as nothing but water diffuses, and hence the total partition of fluid among the tissues will be proportional to these initial rates of osmosis.

It should be mentioned that the values of k and p vary for the same tissue with certain functional changes, such as contraction in muscle, hydrostatic pressure in blood, concentration of the medium for skin, and modification of colloidal states and permeabilities for all tissues.

It is by no means contrary to fact that negative rates of water exchange should occur in tissues that are in dynamic equilibrium with blood. Water is being continually produced or absorbed in the metabolic processes that proceed in every living cell. The rate of liberation or of hydrolysis will depend upon the types as well as the rates of the metabolisms that occur.

SUMMARY

1. Gastrocnemii and upper-leg muscles were placed in various solutions of sodium chloride and gelatin. The initial exchanges of water were measured by frequent weighings, and occurred in proportion to the surface area of the muscle and to the square root of the duration of immersion.

2. Osmosis at different concentrations of sodium chloride was linearly related to concentration except in extreme dilutions, where osmosis was exceptionally rapid. When gelatin was present at any concentration of sodium chloride, the rate of osmosis inward was much reduced. The rates were slightly different at each concentration from those for skinless frogs.

3. From the data for the initial rates of osmosis into each frog tissue that has been studied, osmotic constants are calculated, and the concentrations at which no exchanges of water occur are compared.

4. The initial rates of diffusion of chloride into some of the same tissues are likewise compared by calculating diffusion coefficients.

5. By means of numerical relations, the relative distributions of water among various tissues can be estimated from the initial rates of osmosis manifested upon immersion of these tissues in a common medium. The forces attracting and holding water in tissues are under certain conditions proportional to these initial rates of osmosis.

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EFFECT OF ANOXEMIA, CARBON DIOXIDE AND LACTIC ACID ON ELECTRICAL PHENOMENA OF MYELINATED AND UNMYELINATED FIBRES OF THE AUTONOMIC NERVOUS SYSTEM

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In a previous paper (Heinbecker, 1929) the effects of anoxemia carbon dioxide and lactic acid on the myelinated fibers of the somatic nervous system were discussed. It was there noted that other types of fibers characteristic of the autonomic nervous system were also present in mixed nerves and that the whole subject of the effect of these agents on such nerve fibers would be taken up subsequently.

In the interval, papers by several authors, Davis, Pascual and Rice, (1929) Gerard (1930) and Amberson and Downing (1929) have dealt with the effects of one or more of these agents on nerve. Outstanding has been their support of the conclusions of older investigations, Boruttau and Fröhlich (1904), in which were reported an increase in the total area and duration of the action potential, at least under certain conditions. The authors likewise agreed that a negative retention of the action potential was frequently followed by a positive retention. Their results were apparently presumed to be an expression of changes in form of the axon action potential.

The present paper extends the previously reported findings and conclusions in connection with the fibers characteristic of the somatic nervous system to those of the autonomic system. As in somatic nerves we have found no evidence to indicate a change in duration of the spike of the axon action potential of any of the fiber types of autonomic nerves during depression by anoxemia, carbon dioxide, and lactic acid. Only decrease in amplitude takes place, without change in duration. Increase in negative retention may occur.

In many autonomic nerves we find a group of axons having the approximate time functions of what has been referred to in somatic nerves as the delta wave, (Erlanger and Gasser, 1924) and whose action potentials we now include in the B division of the total potential picture, as the first or B_1 component of that division. They can be definitely shown in some

cases to be afferent axons. For reasons not apparent, further investigation of various nerves (Bishop and Heinbecker, 1930) has failed to support the present position of Erlanger and Gasser (1930) in which they now deem it expedient to disregard the delta wave as an element in the analysis of nerve. A potential with a conduction rate, threshold and absolutely refractory period of the original delta wave or of the B_1 component of autonomic nerves, is invariably present in sciatic nerves of the frog, cat, dog, monkey and man, the distinctness of the potential as a discrete wave varying somewhat in different preparations. If one continues to adhere to the practice of defining the autonomic nervous system as an efferent one, then by such definition the fibers giving rise to a potential with the properties of the B_1 or delta wave are to be classed as somatic, even in visceral nerves.

The two autonomic potential components (B_2 and C potentials), which are present also in somatic nerves, have time functions quite different from those of the somatic fibers, and appear to be assignable to the small myelinated and unmyelinated fibers respectively. (Bishop and Heinbecker, loc. cit.) Figure 1 shows the two fiber types characteristic of the autonomic nervous system in visceral and somatic nerve trunks.

METHODS AND MATERIALS. The technique was in general similar to that used in the study of somatic nerve fibers, the nerve trunks being placed entirely within the gas chamber. The recording mechanism was the cathode ray oscillograph in association with an amplifier yielding up to 200 mm. per millivolt. The characteristics of the amplifier were such as to record the form of the potentials dealt with without significant distortion. The records were made either by single or repeated deflections of the spot across the tube. The speed along the X axis was generally slow enough to permit the use of portrait film for single deflection records.

For experiments in anoxemia commercial nitrogen over 99.7 per cent pure was employed. Pure carbon dioxide and five per cent carbon dioxide in oxygen were the only two concentrations of that gas used. The moistened gases were bubbled through the gas chamber in a continuous stream sufficiently rapid to maintain the desired environment. Lactic acid was applied to the nerve by means of an atomizer giving a fairly uniform concentration of the acid over the entire nerve when desired. As materials, sciatic nerves of bullfrogs, cervical sympathetic and vagus trunks of the cat and turtle were generally used. For special purposes skin nerves of the cat and bullfrog, and limulus nerves to the extremities were studied.

Owing to certain differences in interpretation of our experimental results and those of other investigators using a different type of nerve chamber a word of comment seems advisable. Many have used the type of chamber in which one or more recording electrodes are within the compartment containing the experimental medium and one other on the killed end of the

nerve in another division of the chamber containing normal atmosphere. It has been generally assumed that such an arrangement should eliminate potential alterations at the outside electrodes, due to changes within the chamber. It might minimize certain changes, but it is considered fallacious to hold that it would eliminate them The whole interpolar stretch of the nerve trunk from the point of contact with the proximal lead electrode employed to the distal, acts as a complex unit and complications anywhere along it modify the record of the potential. When the whole nerve is placed within the chamber these complications are not eliminated, but may be amplified to a degree permitting their detection, as explained below.

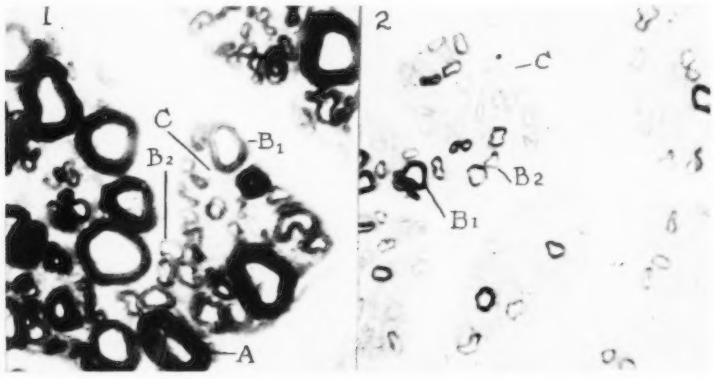

Fig. 1. Cross sections of nerves. Osmic acid x 880. 1—human genito-femoral nerve showing the four fiber types (A, B_1, B_2, and C) essentially responsible for the four components of potential likewise designated A, B_1, B_2, and C. 2—turtle cervical sympathetic trunk showing three fiber types (B_1, B_2 and C) essentially responsible for the three potential components so designated in autonomic nerves.

Results. *Threshold changes during anoxemia, carbon dioxide and lactic acid.* Thresholds were measured by determining the condenser charges required to elicit a just recognizable response. It is essential to note that the thresholds for repeated stimuli even as slow as two per second are often 200 to 300 per cent higher than for single stimuli spaced two or three seconds apart, especially when the nerve is depressed by any of the agents used. Thresholds of both autonomic fiber types were ultimately raised by all three agents (tables 1, 2 and 3). Under anoxemia and in the presence of carbon dioxide there was not infrequently an initial moderate lowering of the threshold of the autonomic myelinated fibers. This lowering of threshold was quite marked with the unmyelinated fibers.

Table 1 is presented to emphasize the fact that the character of the

stimulating current as well as its measure is of importance. This is especially true in anoxemia but is also apparent in the normal. It varies in

TABLE 1

Threshold changes in unmyelinated fibers of turtle vagus nerve, during anoxemia as measured by condenser charges of varying sizes

MINUTES IN NITROGEN	VOLTAGE			RATIO OF VOLTAGES		
	0.01 mf.	0.1 mf.	0.25 mf.	0.01 mf.	0.1 mf.	0.25 mf.
0	24.0	9.0	6.0	1.0	1.0	1.0
15	22.5	7.5	4.5	0.935	0.85	0.75
30	28.5	9.0	6.0	1.2	1.0	1.0
60	45.0	13.0	9.0	1.9	1.45	1.5
75	Not elicited at 160.0	39.0	22.5		4.3	3.8

In the second series of figures the threshold before anoxemia was arbitrarily taken as 1 and other values expressed in relation to it for comparison. Note the small condenser even with high voltage becomes ineffectual in late depression.

TABLE 2

Threshold and conduction rate changes during depression by pure carbon dioxide and recovery in presence of oxygen; bullfrog sciatic

MINUTES IN CO_2		SOMATIC FIBERS		AUTONOMIC FIBERS	
		A	B_1	B_2	C
0	Voltage for threshold response	1.5	6.0	28.5	160.0
	Conduction rate, m.p.s.	35.3	9.5	2.5	0.5
10	Voltage for threshold response	4.5	15.0	51.0	90.0
25	Voltage for threshold response	10.5	16.5–22.0	90.0	90.0
	Conduction rate, m.p.s.	28.0	5.6	1.7	0.24
MINUTES IN OXYGEN					
15	Voltage for threshold response	3.0	16.0	28.5	45.0
35	Voltage for threshold response	3.0-	9.0	25.5	30.0
	Conduction rate, m.p.s.	29.4	8.2	3.0	0.35

degree for the various fiber types. The records indicate that the product of voltage and capacity is less in the range of the small condensers than in

the range of the larger ones, when eliciting threshold responses from the normal nerve. In depressed nerve this product is more nearly equal for large and small condensers. The efficiency of currents of long duration becomes increasingly greater with increasing depression. These findings may first appear to be at variance with those reported by Gerard (1930). However, if one analyzes his results in the critical regions of the curve he publishes it is evident that he has found high voltages with small condensers ineffectual under carbon dioxide depression just as we report. Our results do not cover the later portion of his curve, we not having used condensers of such large capacity.

Changes in conduction rate are presented in tables 2 and 3. It is again noteworthy, as found previously for somatic fibers, that threshold and conduction rate do not always vary in the same direction. Conduction rate is lessened by all of the depressing agents employed.

TABLE 3

Changes in threshold, amplitude of potential, conduction rate and absolutely refractory period, unmyelinated fibers turtle vagus nerve during depression by anoxemia and recovery in oxygen

MINUTES IN NITROGEN	THRESHOLD OF VOLTAGES 0.25 MF. CAPACITY	CONDUCTION RATE	ABSOLUTELY REFRACTORY PERIOD	AMPLITUDE OF POTENTIAL
		m. p.s.	*σ*	*mm.*
0	4.5	0.38	6.7	15.0
15	6.0	0.32	10.0	10.0
30	9.0	0.28	20.0	7.5
45	10.5	0.23	28.0	2.0
MINUTES IN OXYGEN				
30	6.0	0.35	6.7	15.0

Absolutely refractory period changes are indicated in table 3. They are seen to parallel conduction rate changes. Throughout our investigation the absolutely refractory period of nerve has appeared to be the most consistently reliable index of a nerve's condition.

A return to normal on the readmission of oxygen is the rule for all of the above properties of nerve. It was not infrequently found that such recovery was much delayed when washing with physiological Ringer's solution was not also carried out during recovery. The application of such solution always speeded up the recovery process. The threshold for both fiber types may return to a point below the normal. This is interpreted as indicative of a change of nerve reactance in the physical sense, not necessarily as a change in physiological irritability, although in the ultimate

analysis the two are perhaps not distinct functions. In our previous paper it was noted that marked recovery of a nerve axon depressed by carbon dioxide could occur in nitrogen. Similar experiments with autonomic nerve fibers gave similar results. Recovery does not occur after the application of normal lactic acid.

In many experiments definite evidence of treppe was obtained in depressed nerves. On repetition of a constant stimulus the amplitude of the action potential spike would increase. If the stimulation was continued too long, fatigue with depression of the spike and increase of retention would set in.

Order of depression and recovery of the fiber types. The unmyelinated axons are the first depressed to extinction by anoxemia and then the myelinated axons follow. The order of recovery in oxygen is reversed. Somatic myelinated nerve fibers are less readily depressed than autonomic myelinated fibers and they recover first. In the study of the somatic nerve fibers it was noted that the smaller fibers were depressed more rapidly than the larger ones. The order of depression was inversely that of the fiber diameters. The results seemed explainable on a purely physical basis, the ratio of surface area to volume. The autonomic fibers are in general smaller than those of the somatic group. Consequently early depression of them would be expected. The problem is not necessarily so simple, however. The axons of autonomic fibers have properties differing markedly from those of somatic fibers (Bishop and Heinbecker, 1930). Mammalian nerves are more readily depressed than frog and turtle nerves of comparable constitution. The unmyelinated nerve trunks of the limulus are depressed to extinction by carbon dioxide quite readily This does not happen in the myelinated or unmyelinated axons of the vertebrate nerves so far studied.

Fig. 2. Records from turtle vagus nerve to show the effect of pure carbon dioxide on the conducted action potential during 18 minutes of application. Film 1, normal action potential. Films 2, 3, 4, illustrate typical changes during depression. The spike of the second or positive phase of the C potential and its retained portion are depressed with an initial apparent increase in area of the negative spike and its retained portion. There is an increase in the algebraic sum of both phases. This shows some reduction at the end of the period in film 4 where both spike and retained portion are beginning to be depressed. Films 5, 6, 7, 8, and 9 show a reversal of these effects during recovery in the presence of oxygen. Note the increase in negative retention on first recovery. The potential preceding the C potential in these records is the result of the B_1 and B_2 fibers together with the "escape." It is apparent that similar changes are occurring in the B_1 and B_2 potentials. The strength of stimulus used was the same, supramaximal throughout. Films 10, 11, and 12 show the effect of varying strengths of stimuli on the retained portion of the recorded potential. Film 10 is a just threshold C response, supramaximal for B_1 and B_2. Film 11 is a maximal response, film 12 a supramaximal response. Note that there is no change in the retained portion of films 11 and 12 and consequently escape can be ruled out as a factor in determining the form of the potential in these records. Condition of nerve was unchanged during records 10, 11, and 12.

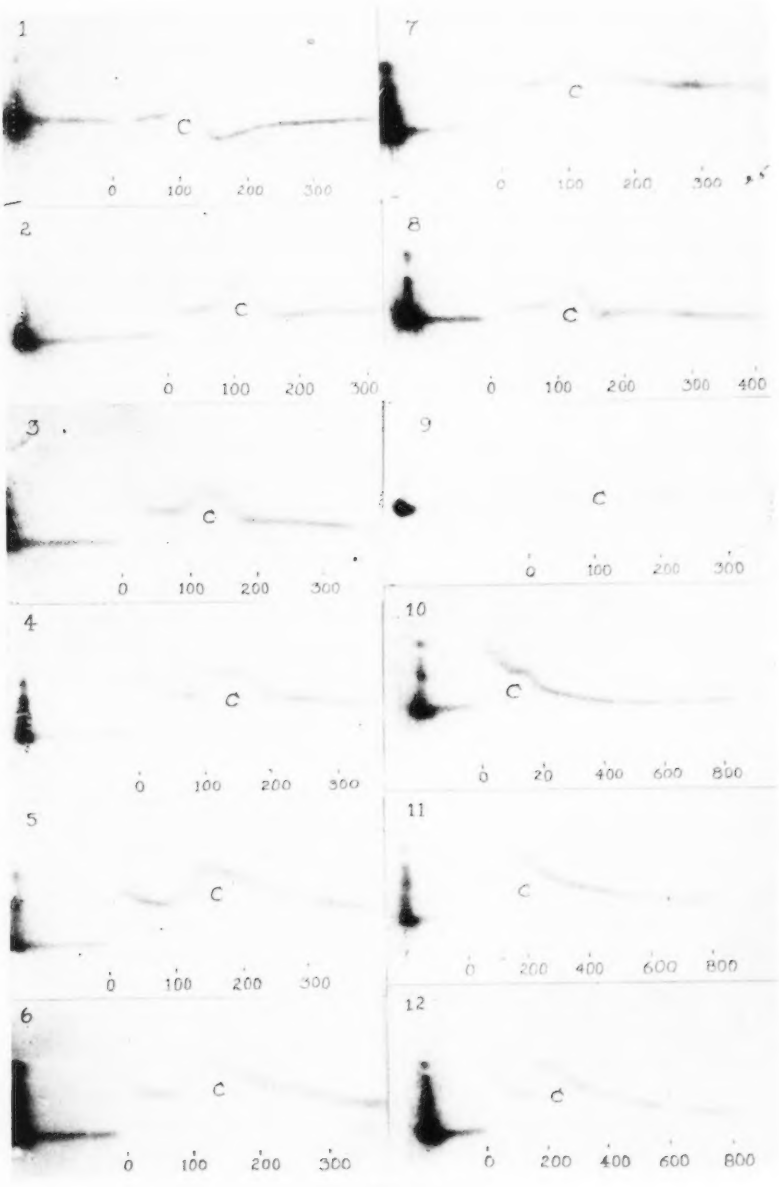
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Fig. 2

Changes in the form of the axon action potential. It has been shown (Bishop and Heinbecker, 1930) that the form of the axon potential of fibers characteristic of the autonomic nervous system differs materially from that of the typical somatic fiber. The rising phase is four to five times as great. Because of the ultimate rise in threshold it is still more difficult to obtain accurate records near the stimulated point throughout the period of depression by anoxemia than in the normal. Experiments carried out with the greatest accuracy possible under the circumstances yield no evidence to indicate that there is any change in duration of the main wave or spike of the axon action potential of autonomic fibers when recorded near the stimulus during depression by anoxemia and carbon dioxide. This is in keeping with the unequivocal results obtained in our previous study of somatic fibers.

To study changes in the so-called retained portion of the action potential, conducted potentials have been utilized for comparison. Inasmuch as there have been no qualitative differences observed with the various agents used in this investigation an experiment in which pure carbon dioxide was used on a vagus nerve of the turtle will be described as typical of one type of investigation employed in this research. Figure 2 illustrates the change in form and duration of the "C" component encountered where the proximal or grounded electrode is on normal tissue at a considerable distance from the cut end. The grid lead is relatively close to the cut end of the nerve which has not, however, been injured otherwise; it is thus a lead from tissue readily susceptible to injury or depression but not from dead nerve. This merely accentuates a condition that always obtains; when the lead is from beyond the killed point, the potential is still diphasic and differential depression still occurs, although the effects are not so plainly observable. It will be seen that in the normal control the negative action potential is followed by a positive phase, and this by a positively retained portion. When the carbon dioxide becomes effective the positive phase and its retained portion are gradually eliminated. With this there is an apparent increase in area of the negative phase, and even in the spike amplitude. The retained portion gradually changes its sign and becomes negative as the second phase is obliterated. Should depression be continued (anoxemic effect) the negative potential and its retained portion will also disappear. It is essential to note that depression in the spike of the action potential does not always go hand in hand with depression in the retained portion. The two ultimately disappear together but first there may be a change in which depression of the spike is associated with an increase of retention, the latter dependent upon the state of the nerve. The experiment demonstrates differential depression of the nerve potential under the two recording electrodes. On recovery in oxygen, spike and retention reappear together. Recovery starts first under the ground electrode and

then follows under the grid. Finally there is again obtained the typically diphasic picture. There is thus also differential recovery under the two electrodes. In some cases an apparent increase in the final maximum amplitude of the spike may occur. This is always associated with some diphasity (incomplete recovery of smaller fibers) which would in itself explain the amplitude increase. Slight changes in the physical resistance of the nerve circuit may also occur. A similar finding was reported for somatic nerve fibers by Heinbecker (loc. cit.).

Experiments were performed where a wide separation of the negative and positive phases of the action potential components was produced by a long distance of conduction between electrodes. Opportunity was thus offered to study the differential effects on the two phases separated. Typical results are recorded in figure 3, 3–12. Differential depression and recovery under the two recording electrodes are definitely established when the two electrodes are on nerve tissue of different viability. In fact, the degree of differential change is directly dependent on such differences of viability. It is noteworthy that the area of retention in normal fresh nerve is very slight even for the "C" potential. It is much less in warm than in cold blooded nerves. Especially marked (film 8) is the increase in area of the positive retention during the early stages of recovery. Inasmuch as recovery of this phase when recording at or near the killed end follows that of the negative phase it is obvious that a first increase in total area of a ballistically recorded action potential would be followed by a decrease, and then with further recovery by another increase.

Lactic acid applied to the recording electrodes produces differential changes similar to those resulting from asphyxia. Weak concentrations applied to the whole nerve cause differential depression. When strong concentrations (n/1) are applied to the whole nerve depression at both electrodes is so rapid that differential changes are hard to observe. Weak concentrations result in a depression of the spike associated with an increase in retention. Strong concentrations depress both spike and retention to extinction. Recovery after such treatment does not occur. Similar differential effects on the negative and positive phases of the potential record can be effected by fatigue when the recording electrodes are on tissue of differing viability (fig. 4). The rate of stimulation to secure this need not be rapid for the unmyelinated fibers (3 to 4 per second). It is somewhat higher for the myelinated autonomics and still higher for the somatic fibers. However, tetanizing currents will bring about the effect in all fiber types. The effect is most pronounced in depressed axons but obtains to a lesser degree in any excised nerve where one electrode is on or beyond injured tissue.

Discussion. The falling phase of the action potential approaches the base line asymptotically and consequently a specific duration is not assign-

able to it. The only measure of duration that would be satisfactory would be the time constant of such a curve. Owing to complications from the diphasic artifacts this curve cannot be obtained in general with sufficient accuracy to make the value significant. The significant measure, therefore, is amplitude. For instance, measurements of the apparent end of the negative retention in partially diphasic records only indicate the point at which positive and negative phases are equal in amplitude and therefore do not signify anything as to the time course of either potential; except that the first retention is steeper than the second if the record crosses the base line. The effect of injury increasing the retention of the second phase will presumably account for the positive retention being visible at all, as in completely diphasic records positive and negative retentions nearly compensate each other, unless the leads are far apart on the nerve.

In our records of nerves the after potentials of autonomic fibers appear to fall off much less abruptly than do those of somatic fibers; that is, the slope of a mathematical curve that approximates them is less. It is certain also that the amplitude relative to that of the spike for the unmyelinated fiber potentials may be greater than for myelinated, and is greater from injured nerve (positive phase record) than from uninjured in general. We are inclined to doubt, however, whether these retentions are of appreciable magnitude in fresh and uninjured nerve, that is, whether such retentions would occur appreciably normally in the body. It is certainly a reasonable inference that they are increased by the process of preparation for experimentation, and that they do not represent a real element of the *action* potential itself. We believe that a reiteration is justified of a previous inference (Bishop 1927) that there are two separate processes involved, only one of which is properly designated as action potential.

Fig. 3. Films 1 and 2. Records from turtle vagus nerve with Wide (30 mm.) separation of the recording electrodes, the grid being near the cut end of the nerve. Film 1 normal; film 2 shows the effect of an atmosphere of pure CO_2 for 12 minutes. Note the depression of the double-humped positive phase of the C potential, allowing the first phase in which the two components have not separated out with conduction to appear longer. Whether the retention of the first phase has altered cannot be determined.

Films 3, 4, 5, 6, 7, and 8. Records from cat vagus nerve with wider (48 mm.) separation of the recording electrodes, but incomplete separation of two phases of a complex C wave, 15 minutes of depression with pure CO_2. Film 3 normal. Film 4 shows slight depression of the spike of the positive phase with some increase in its retention. Film 5 shows an elimination of a positive phase which immediately followed the negative "C" phase on previous records. There is no change in the negative spike except for slight increase in amplitude resulting from a depression of positive phase. There is further depression of the positive phase with further increase in its retention. Film 6 taken after a single stimulus. Film 7 taken after repeated slow stimulation. Fatigue results in a lowering of the positive spike.

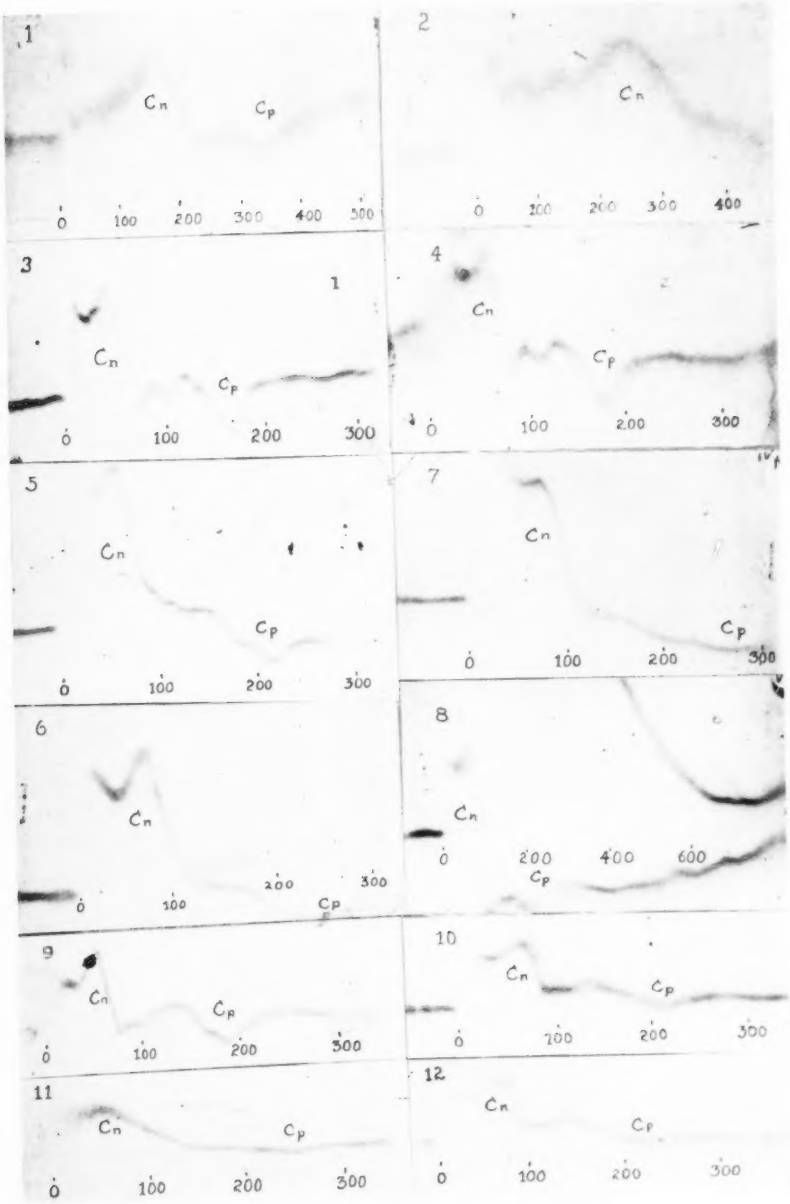
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Fig. 3

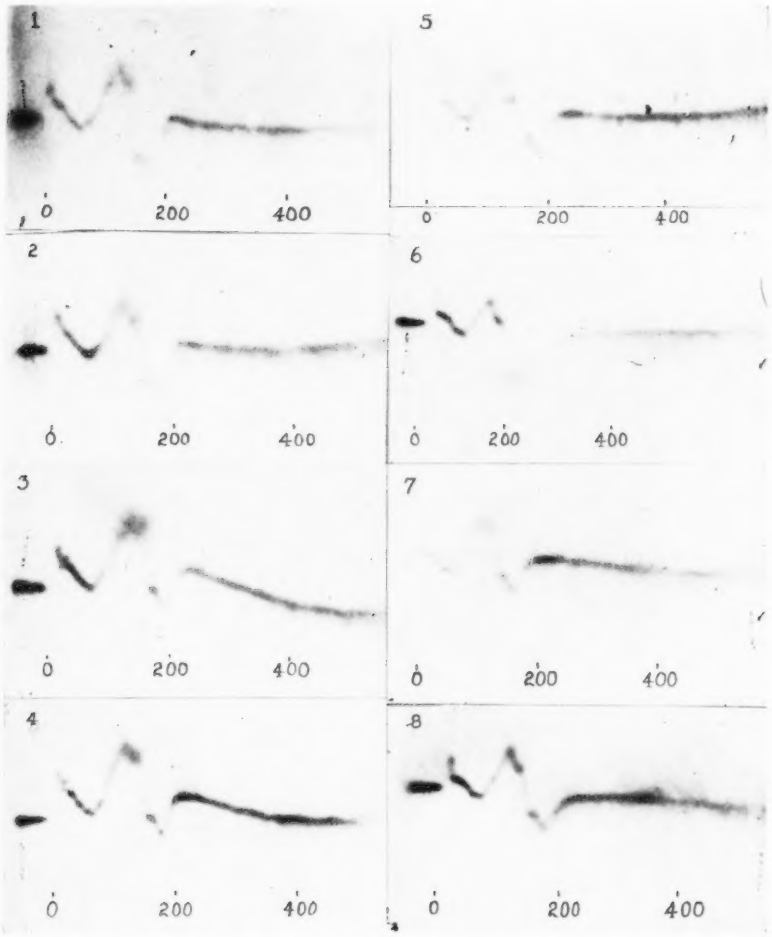

Fig. 4. Records from turtle vagus nerve showing primarily the effect of 5 per cent carbon dioxide on the C potential during 15 minutes of application. Note in films 1, 2, 3, and 4 the progressive decrease in amplitude of the second or positive phase of the C potential. The retained portion of the positive phase increases somewhat. The shift above the base line of the second phase is considered due to changes in physical resistance of the nerve circuit resulting in a change of balance. Balancing of the input bridge permits alteration of the position of the phases with reference to the base line but results in no change in form of the recorded potential. With no increase, even a decrease in the amplitude of the spike of the first or negative phase of the C potential there is an actual increase in area of the total action potential because of the loss of the positive phase of the retained portion. In film 5 recovery in

Five per cent CO_2 seems to have *no detectable effect upon the potential* of uninjured nerve, although the algebraic sum of the two phases, interpreted as the area of *one* phase by authors who have recorded nerve potentials with ballistic systems, may be materially increased. The latter effect also occurs with asphyxia. On the other hand, with concentrated CO_2 rapidly administered, its action may be, and generally is, so rapid upon both injured and uninjured nerve, that while a differential effect is still detectable in the oscillograph record of nerve, the first phase may be depressed practically as soon as the second, and the algebraic sum of the two may never give an area greater than the initial one. In fact, the greater depression of the injured region than of the uninjured, in this event, merely slows down the *apparent* decrease in total area, and thus gives an appearance of slower onset of the depression, even at the uninjured region, than really occurs. The argument applies both to somatic and to autonomic fiber potentials. In recovery, however, the first phase reappears before the second, and always shows a marked increase in amplitude of retention, and apparently also in duration, before the second phase has recovered sufficiently to counterbalance it.

If the nerve is slightly depressed, then even 5 per cent CO_2 may increase the retention, even of the first phase. The logical method for obtaining a *real* increase of nerve potential area with 5 per cent CO_2, therefore, as contrasted with the fictitious increase caused by depression of the second potential phase, is to employ nerve which is in poor condition initially. These two possibilities are difficult to distinguish in records from a slowly deflecting galvanometer, and re-interpretation of published data is therefore difficult.

Quite phenomenal increases in total area of potential, due to CO_2 and to anoxemia, have been reported (Gerard, loc. cit., and Amberson and Downing, loc. cit.). The authors who have reported such increases have always employed leads, in so far as published statements show, that would give definitely diphasic potentials. A nerve killed 5 mm. from the distal

oxygen has taken place. Films 6, 7, and 8 show similar changes, the result of fatigue. Film 6 was taken when only a single stimulus had been applied, film 7 after 20 stimuli spaced at second intervals and film 8 when a single stimulus was applied 10 seconds later than the 20 stimuli applied for film 7. The grid electrode was close to the cut end of the nerve but on live tissue. The condition of the nerve was approximately normal after recovery from carbon dioxide depression. A similar but lesser effect is attainable in an untreated nerve. The effect is one of differential depression at the ground and the grid electrodes.

Film 8 taken early in recovery shows the great magnitude of the positive retention at this stage.

Films 9, 10, 11, and 12. Show the effect of anoxemia on a cat vagus nerve. Film 9 normal. Film 10 shows depression of both phases but relatively more of the positive phase. Film 11 shows almost complete loss of the positive phase with still a definite negative phase remaining. Film 12 shows partial recovery under oxygen.

electrode with fifteen to twenty millimeters between leads, always in our experience gives a markedly diphasic record by the time a record can be obtained. It is obvious that even if the two phases, recorded from two points on nerve, are unequal initially, their algebraic sum may be but a fraction of the simple monophasic potential. If one of them (at the injured end) is now depressed differentially, a large increase of this algebraic sum under depressing agents might be largely due to the fact that only a fraction of the actual area was recorded initially, due to diphasicity of the potential. On recovery from anoxemia especially, the first phase may show recovery of the spike with an exaggerated retention, before the second phase recovers significantly, which would thus give an increase of their algebraic sum when recorded as total potential for two reasons.

However, we do not propose these factors as specific explanations for experiments in the literature not performed by ourselves. When it is kept in mind that such factors as cut branches or idle electrodes between leads affect the record, and that depressing agents will affect such regions differentially, and that even the distance between killed point and lead electrodes affects the character of the record, each nerve arrangement becomes an individual problem, and minor differences in technique have major effects.

Gerard reports (loc. cit.) that under local asphyxial block an initially diphasic potential may become monophasic. We can fully confirm this finding; in fact nerves blocked in this manner give a more completely monophasic picture than by any other technique so far reported. With Gerard's suggested explanation that the monophasicity is due to a more or less complete depolarization of the blocking region, we cannot agree, because not only asphyxia but other blocking agents may give monophasic potentials without significant depolarization of the nerve. This subject will be dealt with more completely in a subsequent publication. It is pertinent to refer here, however, to the differential effects of fatigue in nerves already partially depressed by any agent; the second phase falls out with repeated stimulation, leaving an apparently monophasic picture of the first. This has undoubtedly been an important factor in experiments when tetanizing currents were employed.

SUMMARY

The effects of anoxemia carbon dioxide and lactic acid on autonomic nerve fibers is in general similar to the effects of these agents on somatic nerve fibers.

The threshold is ultimately raised and the conduction rate is lessened. There is usually a marked preliminary lowering of threshold of unmyelinated fibers.

The absolutely refractory period is prolonged. Absolute refractoriness is found to be a most convenient index of the nerve's condition.

Unmyelinated autonomic fibers are depressed more rapidly than myelinated autonomics. Somatic nerve fibers are less rapidly depressed than autonomic ones. The order of recovery in oxygen is reversed.

There is no evidence to indicate any change in the time relations of the spike of the axon action potential. It is lowered by anoxemia, pure CO_2 and lactic acid. The retained portion of the potential is first increased by anoxemia and pure carbon dioxide and weak lactic acid, and later decreased with the spike. It is also increased in depressed or injured nerves by 5 per cent carbon dioxide. Both potentials always finally disappear together and reappear simultaneously.

Since the recorded action potential is in general the expression of a double lead from the nerve's surface, differential depression of the action potential by depressing agents in any method of investigation ordinarily employed is inevitable because of the differential susceptibility of injured as compared with uninjured nerve. The possible effects of differential depression on the ballistically recorded action potential are discussed. The completely diphasic lead is the safest one for interpretation of the functions of nerve.

The effects of fatigue on the recorded action potential are indicated.

Treppe is not infrequently observed in depressed nerves.

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THE UTERUS-OVARY RELATIONSHIP AND ITS BEARING ON THE TIME OF OVULATION IN PRIMATES

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It has been known from time immemorial that sexual union in the mammalia occurs only when the female is in a peculiar "oestrous" or receptive state, a state of behavior in most forms impossible to overlook or mistake, even when the sexes are separated. With the sexes separated, the condition recurs with striking regularity at longer (yearly) or shorter (three to five days) intervals. Careful studies have shown that there is usually a definite relation between oestrum and spontaneous ovulation which is therefore rhythmical.

The sexual behavior of primates does not permit us to recognize definite periods of heat, but here there occurs that regular bleeding—menstruation—which to the present day is obscure in its basic cause or significance.

The discovery of a definite relation between oestrum and ovulation in the lower mammals has naturally led investigators to attempt the correlation of menstruation and ovulation. We will not attempt an historically accurate portrayal of this research. It is enough to state that while some earlier views proclaimed menstruation as identical with oestrum or the preovulation state, the prevailing modern view is that of Robert Schroeder (1928) who insists that ovulation is intermenstrual and, while not as regular as menstruation, is confined with considerable regularity to days 14 to 16 of the menstrual cycle. As will be shown later, Schroeder's data do not establish this conclusion. The aim of the present note is simply to point out that sufficient reliable data exist to demonstrate that primate ovulation occurs at all periods in the menstrual cycle. It is noteworthy that the newer and most accurate data have been furnished by investigators (Schroeder, Corner) who are nevertheless hostile to such an interpretation of it.

The discovery of the method of vaginal smear examinations by Stockard and Papanicalaou (1917) and its immediate extension by Long and Evans (1922), Allen (1922), and others, constituted a new and valuable tool in this type of research. With striking exactitude the time of ovulation could be correlated with changes in the vaginal smear. It was therefore particularly disappointing to find the method inapplicable to the primates,

for the smear changes are not adequately marked here. In the primates we are therefore thrown back on direct histological search of tubes and ovaries for recent or impending ovulation. One needs serial sections of both gonads and the skillful washing of both tubes for the possible recovery of ova, even though the latter procedure is fruitless when ovulation has not occurred. These criteria have been met in the work of George W. Corner who explored histologically the genital tract in a number of individuals of *Pithecus rhesus*, where an accurately recorded, fairly regular menstrual cycle had been obtained. Only five animals were killed during menstruation, two sacrificed on the first day of menstruation and three on the fourth day. Their endometria were dissimilar, one only showing the expected premenstrual growth. His work established beyond doubt the occurrence of menstruation in monkeys possessing a low, extremely simple and intact endometrium, the ovaries being without signs of preceding ovulation. This was a blow to the Hitschmann-Adler-Schroeder dogma of the gradual upbuilding and sudden destruction of the endometrium, which is generally thought to be the cause of menstruation. It is interesting that, faced with these new facts, Corner hypothesized *two types of menstruation*, that with and that without a preceding ovulation. In his four cases he had encountered but one of the first type possessing the high velvety structure we had been taught to view as the typical premenstrual mucosa; the ovary showed a corpus luteum. He encountered four instances of the second type with low endometrium, the ovary showing no recent corpora. He says, "In young mature follicles of *Macacus rhesus* menstruation frequently occurs without ovulation and is therefore not dependent on the presence of a corpus luteum. However, when ovulation occurs, it seems to take place at a definite time, about 12 or 14 days before the onset of menstruation."

In some of the lower mammals, as the rat, guinea pig, and dog, the utero-ovarian cycle normally coincides with exactitude with the oestrous cycle (fig. 1). In these animals changes in the ovary and essentially growth and transformation of the follicles, provoke changes in the entire reproductive tube, from the fimbriated end of the oviduct to the external orifice. Attention was already drawn to these changes (Evans, 1924) which were divided into two groups, those characteristic of prooestrum and oestrum, and those found only after ovulation or postoestrum. The changes consist in growth of the endometrium in the preovulation stage, the period of maturity of the endometrium, and its regression in the later postovulatory stages. The changes proceed pari passu with the growth and rupture or degeneration of the ovarian follicles. It is this series of coincident changes which we designate the utero-ovarian cycle.

In the rat, growth of the uterus proceeds rapidly through prooestrum and oestrum where it reaches its greatest size. Following ovulation the

secretion phase of the endometrium sets in, the so-called "vacuolar degeneration" (Long and Evans, 1922). By the middle of postoestrum (fig. 1) regression has begun and this is completed by the end of that period.

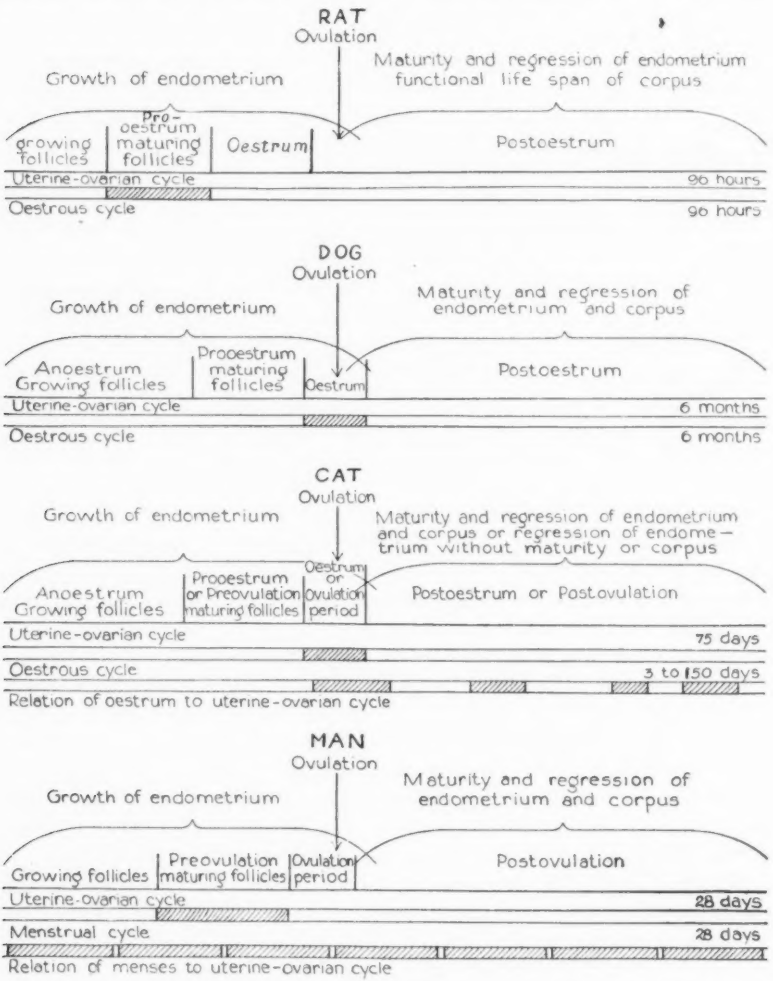

Fig. 1. Diagram showing the relation of the endometrial changes to ovulation. In the rat, guinea pig, and dog, the oestrous cycle coincides with the endometrial cycle. In the cat oestrum may be followed by a number of short recurrent oestrous cycles of irregular length. In man menstruation may occur at any period of the endometrial or ovulation cycle.

In the guinea pig, growth of the uterus begins at about day twelve of the sixteen day cycle and continues for about five days or until after the next ovulation when certain changes occur which mark the secretion phase of the uterine cycle. Regression of the endometrium begins about the fourth day after ovulation.

In studies on the oestrous cycle of the dog (Evans and Cole, 1931) it was found that the new endometrial cycle began in anoestrum. This is shown by a definite resumption of growth of the endometrium (fig. 1). This growth continues throughout prooestrum where it is associated with edema. In oestrum, the height of the endometrium becomes almost maximal. Following ovulation and the formation of corpora, there is further glandular growth but more especially glandular hypertrophy. The length of time during which the endometrium is at its height agrees with the functional period of the corpus and its regression, which begins at about day 20, also agrees with the regression of the corpus. Both growth and regression in the dog are slow processes and the length of time between the end of regression and the beginning of growth is apparently a very short one, leading us to question whether the genitalia are ever at rest.

In the cat, if we exclude the phenomena of recurrent oestrum, similar conditions are found (Evans and Swezy, 1931a). In the beginning of growth the endometrium is very low with a few straight glands. As in the dog, growth of the endometrium begins before any definite evidences of maturing follicles are found in the ovary, though abundant small and medium-sized follicles are present in both animals, many of which may be atretic. Growth in which the glands participate continues until, at the time of ovulation, the height of the endometrium is about as great as that associated with the presence of a corpus in the ovary. The glands are complex, more so than in the dog, though slender, with small cells and having the same appearance as in the earlier growth stages. From this time on, two distinct phases are presented by the uterus, dependent on whether ovulation has occurred (fig. 1). When this is the case and corpora are present, the glands of the endometrium continue to increase in complexity with great hypertrophy of the cells. When ovulation does not occur, the mature follicles persist for a few days, and growth, as shown by the presence of mitoses in the cells of the uterine glands, continues until the follicles become atretic, following which, regression of the endometrium sets in. There is thus in the cat, a very definite relation between the growth of the endometrium and the presence of maturing follicles in the ovary, even when these do not rupture.[1]

[1] It must be pointed out that follicular size cannot be taken to constitute definite evidence of maturity, since in the guinea pig (Evans and Swezy, 1931b), follicles of preovulation size are found as early as day ten of the cycle, while growth phases in the endometrium do not begin until about the twelfth day.

In our opinion, the essential note furnished by the elaborate studies of Schroeder (1928) on the human endometrium, concerns the striking agreement of the cyclic endometrial changes with the changes in the ovary. A very rapid growth of the endometrium occurs after the period of degeneration, occupying about six days. By the time ovulation occurs the endometrium has nearly reached its full height, though a slower growth continues for a few days. Following ovulation, evidences of glandular secretion with an increasing complexity in the glands themselves occur, such as is associated only with the presence of a corpus in the ovary.

In each of these forms therefore (rat, guinea pig, dog, cat, man) growth of the endometrium ceases entirely a few days after the ovulation period, whether ovulation has occurred or the mature follicles degenerate. A diagrammatic presentation of this is given in our figure 1. The presence of active growth in the endometrium without evidences of secretion or enlargement of the cells or great complexity in the glands is, in these animals, indicative of the ovulation or preovulation period and has been found at no other time of the cycle. Experimental work has shown that this results from specific hormones (folliculin, oestrin). Likewise a regressing endometrium normally occurs only after an ovulation period has passed. The importance of this concept in the study of animals like the primates, in which no external signs of ovulation have been established, is at once evident. A study of the uterus should show whether the "ovulation period" is about to occur or whether this has already passed, even though it did not result in follicular rupture. This would seem to be a more dependable method than a study of the ovary, since there is no way to distinguish a ripe follicle until it actually bursts, nor is there any certain way to prove that an atretic follicle is one that has fully matured without ovulation, as in the cat, or is merely one of the number of follicles which become atretic in every cycle.

As an application of this, we may venture to apply these criteria to the monkeys given in Corner's paper (1923), his plate 4 lending itself admirably to this purpose, as the excellent photographs of the uteri of these monkeys may be directly compared with photographs of the uteri of the dog and cat. His figure 34 shows the end of regression of the endometrium, and the beginning of growth is shown in succesive stages up to the ovulation period in figures 33, 30, 32, 35, 28, 31, 29, and 25, in the order named. Comparing these figures with those of the dog and cat it is found that the different growth phases are identical with those found in the endometrium of these animals in the preovulation stages, both as regards height of the endometrium and development of the glands. Figure 29 still falls short of the maximal preovulation development and this interpretation is supported by the fact that no large, healthy follicles were present in the ovaries, though atretic ones were found. Again judging by similar conditions in

the cat and dog, it seems fairly certain in this case that with the lapse of a few more days, possibly three or four, some of the medium follicles would have attained maturity and the ovulation period would have arrived. Since this condition was found on the nineteenth day of the menstrual cycle, its ovulation period would therefore have been reached shortly before the end of that cycle.

The careful work of Corner, Allen, and Hartman, has disclosed clear instances where neither ovary possessed recent corpora although the animals were in apparent health and the menstrual bleeding had been regular. It is apparent ovulation itself can be "skipped" for one or more cycles. This is frequently the case in the cat where coitus has not been allowed. Nevertheless in such instances the same characteristic preovulatory growth changes occur in the endometrium, and these are followed by a distinct regression phase when the mature follicles become atretic. The

TABLE 1

Data on conditions in the endometrium in 898 human cases with regular menstrual cycles. After Schroeder, 1928

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Beginning of proliferation	1	1	2	5	8	11	17	6	5	7	6	5	5	3	2	2	2		2	1	1	2		3		1		2
Middle of proliferation			1	3	8	10	13	29	30	38	24	8	5	5	5	1	2		1	1		2	1	2				2
End of proliferation					2	1	1	2	1	9	12	25	19	22	11	16	6	10	1	4	4	3	3	3		2	2	
Beginning of secretion								2		5	4	3	5	11	23	9	29	15	26	29	13	6	6	5	2	5	4	7
Middle of secretion									1	1			1	3	4	4	6	4	5	15	18	17	23	14	9	4	3	8
End of secretion																	2		1	2	2	3	4	8	19	7	2	12
Desquamation	2	3	1	3	3	3	1		1	1	1				1					1		1		1	2		1	4
Regeneration				3	1	1	2		1				1															1
	3	4	4	14	22	24	33	39	39	61	47	41	36	44	45	33	47	29	36	53	38	34	37	36	32	19	12	36

condition of the endometrium thus gives a clear indication of the phase of the ovarian cycle.

In his Veit Handbuch chapter (1928) Robert Schroeder listed 898 human cases with regular four-weekly cycles, describing the endometrium in each case. He recognizes eight types of endometrium, the beginning of proliferation, the middle of proliferation, the end of proliferation, the beginning of secretion, the middle of secretion, the end of secretion, desquamation, and regeneration. These endometrial types can be satisfactorily compared with the stages of the endometrial cycle in the dog and cat, stages which are definitely related to ovulation or the "ovulation time." When the comparison is made, it is evident that ovulation must occur during the third period, the end of proliferation. But endometria showing this stage of development he finds occurring from the fifth to the twenty-seventh day of the menstrual cycle, and only the majority between the tenth and eighteenth days. Seven cases out of thirty-six examined at the twenty-

eighth day showed the beginning of secretion, a phase immediately following ovulation and the formation of a corpus. A few cases of desquamation were scattered through the entire cycle. His table (reproduced in table 1) shows that the largest number of ovulation periods for an equal time interval occurred between the tenth and eighteenth days. It also shows very definitely that what we have called the "ovulation time" (regardless of whether ovulation actually occurs or not) was found at all periods of the menstrual cycle. Thus in 157 cases examined at days five to nine, ten showed the phase associated with ovulation, and in 135 at days twenty-five to twenty-eight, thirty showed the same phase (table 2, uterus).

Of interest in this connection is the work of Newell, et al. (1929), who have examined 90 human cases at all stages of the menstrual cycle with the greatest number between the twelfth and sixteenth days, and, in those presenting evidences of fresh corpora, washing the tubes for ova. Ova

TABLE 2

"Time for ovulation" as shown by the complexity of the endometrium and abundance of ova

DAYS OF MENSTRUAL CYCLE	UTERUS—SCHROEDER, 1928		OVARY—EVANS AND SWEZY, 1931	
	Cases showing ovulation time	Number of cases examined	Cases showing ovulation time	Number of cases examined
5–9	10	157	3	5
10–15	149	274	14	29
16–23	180	307	12	33
24–28	30	135	8	11

were recovered in four cases, one on the fourteenth, two on the fifteenth, and one on the sixteenth day.

In the 90 cases more than 30 presented evidences of fresh corpora judged by gross examination, a "few" of which however on histological examination proved to be "fairly well developed." Of these 30 cases the time of the operation with reference to the menstrual cycle is given in only 14 cases in which it occurred from the twelfth to the sixteenth day. This would lead us to suppose that the other 16 occurred at other days of the cycle. In any attempt to designate a special time for ovulation incomplete data cast doubt on the conclusions drawn, as in this instance, where the histories of more than half of the cases are omitted from the record. It is evident that finding a "fairly well developed" corpus at day sixteen, for example, or a very recent corpus at day eight, would have a definite bearing on the question of the time of ovulation, even though no ova ere recovered. Finding four cases of actual ovulation out of a total of 90, still leaves 86 to be accounted for. Fourteen cases of recent corpora on days twelve to sixteen out of 90, falls below the percentage we could

reasonably expect for these days, judging by the data given in our tables. The authors' criticism of some of the earlier records of the findings of human tubal ova (Letheby, Hyrtl) is partly based on their belief that "it is now known that ovulation usually occurs at some time during the intermenstrum," days which they estimate at between days eleven and fourteen of the menstrual cycle.

During the World War, German gynecologists recognized the unusual opportunity to study the incidence and length of pregnancies which could be dated from a single copulation—in these instances resulting from cohabitation during very short military furloughs of the husbands. In the data thus accumulated it is clearly shown that pregnancy may follow coitus at any time in the cycle, the expected menses being prevented. The results of these studies (table 3) agree broadly with the data given by Schroeder.

TABLE 3

Time of conception given by German investigators, after Asdell, 1927

AUTHORITY	1-9 DAYS	10-14 DAYS	15-23 DAYS	TOTAL
Siegel	44	13	23	80
Nurnberger	80	56	47	183
Jaeger	48	22	29	99
Heeker, Buhl and Schlichtling	175	94	121	398
Furst	58	51	63	172
Total	405	236	283	922

It has been the custom of clinicians to date the beginning of pregnancy from the time of the first missed menstrual period or a certain arbitrary number of days before that period. It has been found that while the duration of pregnancy may vary within wide limits, yet, in the majority of cases, this variation does not exceed one lunar month. This difference falls within the time limits of a single menstrual cycle, with the possibility of ovulation, hence conception, at any time in the cycle.

A further confirmation of these results is obtained by a study of ovogenesis and the formation and growth of ovarian follicles. It has been found in the lower mammals that the period of greatest abundance of ova and follicles occurs at a time preceding ovulation, with the degeneration of most of these at the time of ovulation. Similar conditions are found in the human ovary. It is significant that, just as Schroeder has found all stages of the endometrium at all stages of the menstrual cycle, so also these periods of great abundance of ova have been found at all stages of the menstrual cycle, the greatest number admittedly occurring near the middle of the cycle (table 2, ovary). The number of our ovarian studies

shown in table 2 is small when compared with the Schroeder uterine studies, yet the results are similar. Our cases include a few in which a newly-formed corpus was present. A fuller discussion of the relations of ovulation to the general ovarian picture and the follicular or ovulation cycle will be found elsewhere (Evans and Swezy, 1931b).

The importance of the convincing demonstration by Corner (1927) that menstruation may occur in the monkey without desquamation of the uterine mucosa,[2] has already been referred to. As he has pointed out, this means a revision of the prevailing theory of the cause of menstruation. Hartman (1930) has recently experimentally provoked menstruation in mature and infant monkeys with hypophyseal hormones. He specially states "bleeding from the uterine mucosa is a phenomenon independent of hyperplasia, swelling, or even congestion of the tissues." But a careful review of the literature indicates that Corner's fine discovery and this conclusion from Hartman's beautiful work in the monkey has already been established for man (i.e., menstruation occurring with little or no destruction of uterine tissue). Of Schroeder's 7 cases examined during the first two days of menstruation 2 showed a low intact endometrium, designated by him "beginning of proliferation." Nor have these facts escaped other observers as reports to this effect have been given by De Sinety (1881), Oliver (1906, 1920), Christ (1892), Moericke (1882), and Lipes (1904). In these cases menstruation occurred with little or no destruction of uterine tissue.

What we have to face is that the uterus-ovary morphogenetic cycle thus appears to occupy a 28 day or menstrual interval, but that it is by no means accurately related to the bleeding cycle. Hartman's (1930) experimental work confirms this but he believes that normally there is a definite relation of the two phenomena, accepting for an explanation of this the Hitschmann-Adler-Schroder dogma, thus, "normally in women and during the breeding season in apes the follicle stimulating and the luteinizing hormones come into play, ovulation occurs, the anterior lobe is stimulated, and bleeding occurs from a 'prepared' or pregravid endometrium of the Hitschmann and Adler type."

In fact a study of table 1, giving the results of Schroeder's careful work, gives ample proof that menstruation may occur with the endometrium at all stages of growth, maturity, and regression. Since this is the case beyond further doubt, it is evident that the arbitrary placing of the time of ovulation at any definite point in the menstrual cycle is indefensible.

Various attempts have been made by proponents of the theory that ovulation occurs at a definite time in the menstrual cycle, to explain the occurrence of conception at other periods of the cycle, none of which,

[2] B. Sutton was the first to describe this in his excellent paper in 1886.

however, have any actual proofs in support of them. Among these may be mentioned the theory that copulation itself may bring about rupture of the follicles even though the normal period for that has passed. Conditions in the cat, however, show that mating has no effect on the follicles unless they themselves are at or near the ovulation period. There is no record in our own investigations of hundreds of animals, or in the literature with which we are familiar, which shows that ovulation has ever occurred at any other time in the endometrial cycle than just before the completion of the growth phase of the endometrium. There is a basic physiological reason for this since the growth of the endometrium must occur before implantation of the ovum is possible. The relation between the ovarian and endometrial cycles is a most intimate and, in our experience, an invariable one.

Schroeder's interpretation of his results does not agree with that given here. He believes that in those cases where ovulation occurred near the beginning of the cycle, the following menstruation would have been speeded up to bring it within the accepted time limit for the regression of the corpus and endometrium,[3] his view of the relation of these to menstruation being that of cause and effect. Also, those cases in which ovulation occurred near the end of the cycle would have a delayed menstruation period, though he lists all of these cases as having a regular four-weekly cycle. He makes no attempt to explain the correlation between the last menstrual period and many of the cases, as, for example, those cases in which the beginning of proliferation, which he places immediately after menstruation, begins at days ranging from 7 to 28 in the cycle. He believes that ovulation normally occurs on days 14 to 16 of the menstrual cycle, but a glance at his table will show that equally large numbers of new corpora, indicated by the beginning of secretion, occurred on days 17 to 21. In fact, out of the 368 cases in his table which show the "ovulation period" (end of proliferation and beginning of secretion) only 92 or 25 per cent come at days 14 to 16. Furthermore, out of 25 cases examined during menstruation (days 1 to 4) only twelve showed the typical menstrual picture, the remaining 13 showing proliferation. These facts invalidate the conclusions he has drawn regarding the relations of menstruation and the time of ovulation.

Schroeder concludes that ovulation occurs in every cycle *and his data seem to bear this out*, since functional corpora were present in nearly half of his cases, the other cases showing the preovulation stage of the endometrium. He further states that maturing follicles were present in every case showing proliferation, and a corpus was present in every case showing

[3] "In diesen jedoch würde entsprechend der schon zu weit vorgeschrittenen Phase bei verfrühtem Ovulationstermin auch die nächstfolgende Menstruation entsprechend früher kommen, während bei verzögertem Ovulationstermin auch die Menstruation entsprechend später einsetzen würde."

the secretion phase without exception. This again indicates a consistency in the uterus-ovary relationship that cannot be questioned.

The number of monkey ovaries examined with no corpora present seem to indicate that, in this animal, as in the cat, the mature follicle may sometimes degenerate instead of ovulating. Such a condition we feel could be determined by an examination of the endometrium. As in the ovary there seems to be no resting phase in the uterus. Regression proceeds quickly in man, very slowly in the dog, slowly in the cat, and this is followed by the beginning of slow growth which reaches its highest point at about the ovulation period, though the greatest degree of complexity in the non-pregnant cycle comes only with the development of the corpus. This cycle thus characterized by regular growth stages and regression in a single tissue (endometrium), gives a series of events by which the gonadal cycle may be determined. When the ovulation period is reached without actual rupture of the follicle, as may happen in the cat, a definite record of this is found in the endometrium. Without such a study of the endometrium these follicles might be passed over as the atretic follicles which are ordinarily found during the latter part of every cycle, or vice versa.

There are no compilations of data known to us which suggest results differing from those given in the tables appended herewith. None of the four different lines of approach to this subject, the condition of the ovary, the condition of the uterus, the length of pregnancy, or the time of conception, justifies us in assigning ovulation to an invariable period of the menstrual cycle even though the period be broadly defined. In particular the conception of the uterus-ovary relationship given herewith enables us to utilize differently Schroeder's careful histological study of hundreds of endometrial scrapings, and to conclude that ovulation, while more apt to occur in the mid intermenstrum, occurs at all times in the human menstrual cycle.

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EFFECTS OF POSTERIOR PITUITARY EXTRACTS ON BASAL METABOLISM[1]

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Studies of the effect of posterior pituitary extracts on basal metabolism have given inconsistent results. After the administration of these substances an increase in the metabolic rate has been noted by Bernstein and Falta (1918), McKinlay (1921), Zloczower (1923), and Nitescu and Gavrila (1929). A fall has been noted by Klein, Müller, Scheinert, and Steuber (1923), Lambie and Redhead (1929), and Cushing (1930). Experiments of Weiss and Reiss (1923) on rabbits indicated an increased oxygen consumption when food was given but a slightly decreased metabolism in prolonged fasting. The results on the respiratory quotient also varied greatly.

These inconsistencies in the literature may be due to the fact that the doses administered were too small to give definite results or that the extracts used contained varying amounts of the oxytocic and pressor principles which might have different effects on metabolic rate. Hence, we have repeated the previous work with varying dosages of α and β hormone as well as with pituitrin.

METHOD. The oxygen consumption, carbon dioxide production and respiratory quotient were obtained by Haldane's (1892) method on male rats (weighing 270 to 454 grams) which had been fasted approximately 24 hours previous to each experiment.

The experimental animal was placed in a wide-mouthed, rubber-stoppered bottle the temperature of which was maintained between 24.6° and 28.9°.[2] To remove carbon dioxide, two pairs of 6 cm. U-tubes were used, the first of each pair containing Wilson soda lime and the second containing pieces of pumice stone saturated with concentrated sulphuric acid. Between the carbon dioxide absorbers and the animal chamber two more tubes containing pumice were inserted to remove the moisture liberated from the rat chamber. Dry carbon dioxide-free air was drawn through the chamber and the absorber at the rate of about 400 cc. per minute. Various tests showed that this system was capable of absorbing all the

[1] A preliminary report appeared in the *Proceedings of the Society of Experimental Biology and Medicine*, 1930, xxvii, 815.

[2] According to Houssay the metabolic rate of rats is at a minimum between 25° and 30°C.

moisture and carbon dioxide if the soda lime and acid were frequently changed. During weighings the tubes and the animal chamber were kept tightly closed by small pieces of glass put into the ends of the connecting tubes.

The apparatus was weighed in three parts: *1*, the animal chamber; *2*, the two water absorbers connected with this chamber, and finally, *3*, the carbon dioxide absorbers. The gain in weight of *3* is due to the carbon dioxide produced, whereas the increase in weight of the whole system is caused by the oxygen consumed. The respiratory quotient may be calculated from these values by the following equation:

$$\text{R.Q.} = \frac{\text{weight } CO_2}{\text{weight } O_2} \times \frac{32}{44}$$

The gaseous metabolism was measured in one hour periods. After two or more preliminary periods to determine the basal oxygen consumption the rat was injected with pituitary extract, replaced in the chamber and weighed. The rat usually lay quiet except for some restlessness in the first 8 to 10 minutes after injection and infrequent washing movements thereafter.

The apparatus was tested by determining the respiratory quotient of burning alcohol and a value of 0.686 was obtained. It is probable that the results in the actual experiments were more accurate than those obtained on alcohol. In the latter case in order to support combustion the air had to be drawn through at a very rapid rate. The differences between successive determinations of the preliminary basal oxygen absorptions yielded a variation of ±5 per cent from the average. These variations were not due to errors in weighing, since the balance used was accurate to 3 mgm. and the oxygen absorption was of the order of 500 mgm. It can be seen that the larger part of the deviations was probably due to changes in the oxygen consumption of the rat.

For the subcutaneous injections, pitressin (1 cc. containing 10 pressor units), pitocin (1 cc. containing 10 oxytocic units) and "surgical" pituitrin (1 cc. containing 20 pressor units and 20 oxytocic units), were generously supplied by Parke, Davis and Company. When desired, these extracts were freed of chloretone by evaporating to one half their volume and then making up to volume again with sterile water as was suggested by Dr. Oliver Kamm.

Results. The range of variation of the results and the average change after the injection of each of the extracts are presented in tables 1 and 2.

Pituitrin. Experiments in which large doses (23 to 66 units per kilo) of chloretone-free "surgical" pituitrin were injected, disclosed a fall in the oxygen consumption of 19 per cent in the first hour after injection. This effect continued to a slight extent in the second and third hours. With

these doses there was apparently no relationship between the size of the dose and the decrease of metabolism.

Pitressin. The results with pitressin seemed to indicate that the decrease in metabolism obtained with pituitrin was due mostly to the pressor fraction.

A. Small doses. The first experiments were performed with pitressin which contained 0.5 per cent chloretone as a preservative. With doses from 11 to 17 units per kilo, a slight rise was indicated by an average of 11 per cent above the basal oxygen consumption. As will be considered below, this result is probably due to the method of administration and not to the pitressin itself. One experiment not included here, in which the pitressin was given intramuscularly instead of subcutaneously, a fall of 27 per cent in the first hour and of 14 per cent in the second and third hours

TABLE 1

Changes in oxygen consumption after injection of pituitary extracts

SUBSTANCE INJECTED	NUMBER OF EXPERIMENTS	UNITS PER KILOGRAM	BASAL O_2 CONSUMPTION	CHANGE FIRST HOUR AFTER INJECTION		CHANGE SECOND AND THIRD HOURS AFTER INJECTION	
					Average		Average
			mgm. per hour	*per cent*	*per cent*	*per cent*	*per cent*
Pituitrin (chloretone-free)	6	23–66	418–642	−6 to −33	−19	+11 to −29	−6
Pitressin (chloretone)	11	11–17	349–531	+34 to −13	+11	+20 to −17	+4
Pitressin (chloretone-free)	13	17–40	363–542	+30 to −25	−1	+24 to −16	+5
Pitressin (chloretone)	6	18–27	375–489	−5 to −34	−18	+6 to −12	−2
Pitressin (chloretone-free)	5	50–100	435–527	−17 to −34	−22	−8 to −13	−10
Pitocin (chloretone)	4	14–80	357–438	+20 to +4	+14	+19 to +4	+10
Pitocin (chloretone-free)	7	2.5–80	374–502	+64 to +16	+32	+24 to +5	+14
Water	9	0.5–2.5	392–535	+32 to +9	+17	+14 to −2	+7

was observed. This would seem to indicate that the injection was more effective when given directly into muscle. Since it was suggested that chloretone might have a depressant action, another series of experiments was performed with chloretone-free preparations.

Small doses (17 to 40 units per kilo) of chloretone-free pitressin yielded variable results, although the average was close to the basal. In order to produce a marked effect, larger doses were given.

B. Large doses. With large doses (18 to 27 units per kilo) of pitressin containing chloretone, a fall which was roughly proportional to the dose (average 18 per cent) appeared in the first hour after injection. These animals injected with pitressin containing chloretone showed signs of depression (stupefication and cyanosis) which were hardly apparent with larger doses (50 to 100 units) of chloretone-free pitressin, doses which caused a similar fall (average 22 per cent) in the oxygen consumption.

Pitocin. A few experiments, in which pitocin containing chloretone was used, yielded a rise in oxygen consumption of 14 per cent. This rise was less marked with the largest dose, probably because of the depressant effect of the chloretone.

In other experiments in which chloretone-free pitocin was used the oxygen consumption increased in the first and possibly in the second and third hours after injection. In two experiments, not included here, the metabolism was not measured for 15 to 20 minutes after the injection in order to allow the rat to recover from any effect of movement in the first few minutes. These experiments show practically no rise in the first hour after injection so that it would appear that any rise due to pitocin comes soon after the injection.

Effect of injection. In order to determine how much of the rise caused by pitocin might be due merely to the effect of injection, 0.5 to 2.5 cc. of water

TABLE 2

Average respiratory quotients after injection of pituitary extracts

SUBSTANCE INJECTED	NUMBER OF EXPERIMENTS	UNITS PER KILOGRAM INJECTED	R.Q. BEFORE INJECTION	FIRST HOUR AFTER INJECTION	SECOND AND THIRD HOURS AFTER INJECTION
Pituitrin (chloretone-free)	6	23–66	0.731	0.781	0.736
Pitressin (chloretone)	18	11–27	0.732	0.762	0.730
Pitressin (chloretone-free)	18	17–100	0.732	0.767	0.732
Pitocin (chloretone)	4	14–80	0.728	0.790	0.724
Pitocin (chloretone-free)	9	25–80	0.734	0.755	0.727
Water	9	5–2.5 cc.	0.734	0.743	0.728

were injected and a significant rise in the oxygen consumption (17 per cent) was obtained. The rise due to pitocin (32 per cent) is, however, significant even when the amount due to injection (17 per cent) is subtracted, since an increase of 15 per cent still remains. The same effect of injection is also suggested by the fact that the change produced by pitocin is not related to the size of the dose, a very small dose still showing a rise. Thus any rise in metabolism in the first hour after injection is really less than it appears by approximately 17 per cent, whereas any apparent fall is actually greater by the same amount.

Respiratory quotient. A summary of the results on the respiratory quotient appears in table 2. With all the substances injected, except water, a slight rise (from 0.728–0.734 to 0.755–0.790) was indicated.

Discussion. The results indicate that injections of pituitrin diminish the metabolic rate and that this action is due to the pitressin fraction, since pitocin increases the oxygen consumption to a slight extent. In this con-

nection it is interesting to consider the possible mechanisms by which each of these substances may produce its effects. The decreased oxygen consumption observed with pitressin may be due either to effects on brain centers or to peripheral vascular changes. The central site of the action of pituitrin has been demonstrated by Raab (1926). Cushing (1930) has recently injected pituitrin directly into the cerebral ventricles of a patient and before the hormone could have diffused to the blood stream, noted the beginning of a great fall of temperature. It is therefore probable that pituitrin has a direct effect on centers regulating temperature and thus increases heat loss and diminishes heat production. In Cushing's subject, loss of heat was indicated by profuse perspiration, while decreased heat production was evidenced by a fall in metabolic rate from −27 to −50 per cent.

In addition to probable vasomotor reactions attendant on a loss of heat from the body, there are other vascular effects associated with constriction of the arterioles. This action has been shown to be due primarily to pitressin (Rowe, 1929; Hjort, 1928; Hemingway and Peterson, 1929) and has been studied in the lung, heart, and kidney. The experiments of Gruber (1929) and Gruber and Kountz (1929) indicate that pitressin causes an initial fall in blood pressure and a slowing of the heart rate, due to an asphyxia caused by constriction of the coronary vessels. This work has been confirmed by Clark (1929) who concludes that death from pituitary pressor extract is due to cardiac depression and not to respiratory failure. With insufficient blood supply to the various viscera, a diminution in metabolic rate of the entire organism may be expected. Apparently the decrease in oxygen consumption is produced by the same processes which in larger doses cause death. Whatever is the exact cause for the diminished oxygen consumption this phenomenon may be followed by a rise in the level of the blood lactic acid, since less energy is available to dispose of lactic acid. Such a rise has been observed after injections of pituitrin and especially pitressin (Himwich and Fazikas, 1930).

The rise in metabolism due to pitocin is more difficult of explanation. Pitocin apparently has no action on the blood vessels. Moreover, the most marked effect of this substance, the effect on uterine muscle, is eliminated by the use of male rats. The storage of fat in liver observed by Coope and Chamberlain (1925) and Oshima (1929) has not been substantiated by Van Dyke (1926). However, Raab (1926–27) thinks that pituitrin causes an increased oxidation of liver fat. In addition to actions in the liver there are others in the blood. Pituitrin (Raab, 1926) and pitocin and pitressin (Himwich, Haynes and Spiers, 1930) cause a decrease in blood fat. It is therefore possible that the augmentation in oxygen consumption following the injection of pitocin may be imputed to the extra work of the organism in connection with the changes of fat in liver and blood, whereas in the case

of pitressin, this effect is completely overcome by those of the central and vascular changes.

Two processes may be active in producing the rise in respiratory quotient observed in the present experiments. There may be an increased oxidation of carbohydrate as well as a liberation of extra carbon dioxide. Pituitrin and particularly pitressin (Himwich and Fazikas, 1930) cause an accumulation of lactic acid in the blood. This lactic acid in turn displaces carbon dioxide from bicarbonate, causing a rise in the respiratory quotient.

As a result of the present work there are two possible causes for the discrepancies in the literature. In the first place, due to the opposing effects of the oxytocic and the pressor principles on metabolic rate it is quite likely that preparations containing varying amounts of the α and β hormones would yield opposite results. Nitescu and Gavrila (1929), who were the only observers to use both principles, found an average rise of 10 per cent with pituitrin (10 units) and β hormone (10 units) and no significant change with the α hormone (10 units).

More important is the amount of pituitrin injected. With the dosage used by most observers we could obtain no definite results. The reasons for the larger doses necessary to produce changes in metabolic rate when pituitrin is injected peripherally become apparent in the light of the work of Raab (1926), who showed that pituitrin injected into the ventricle had more potent effect than when given subcutaneously. Cushing (1930) was unable to obtain a fall in metabolic rate when pituitrin was injected either subcutaneously or intravenously, although the oxygen consumption decreased when the hormone was injected into the third ventricle. Finally, it may be pointed out that a central site for the action of pituitrin makes for the possibility that the comparativey small amount of that hormone found in the cerebro-spinal fluid may nevertheless have a distinct effect on metabolic rate.

SUMMARY AND CONCLUSIONS

In 64 experiments on 20 rats the changes in oxygen consumption, carbon dioxide production and respiratory quotient after the subcutaneous injection of pituitrin, pitressin, and pitocin, were determined by Haldane's method.

It has been observed that pituitrin decreases the metabolic rate. This action of pituitrin is due to pitressin and occurs despite the effect of pitocin, since pitressin markedly diminishes the metabolic rate, while pitocin increases it slightly, but definitely. Such opposing actions may explain some of the inconsistencies in the literature since the different preparations used by various observers may have contained varying amounts of the oxytocic and pressor principles. Moreover, some of the previous workers

injected pituitrin peripherally in doses that were too small to produce definite changes, since in the present experiments effects on metabolic rate were observed only with large doses.

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A STUDY OF BRONCHIAL REFLEXES IN THE GUINEA PIG

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There is a growing belief that a large number of cases of asthma cannot be explained on the basis of sensitization to foreign proteins. In the etiology there remain to be considered reflex spasm of the bronchioles and other as yet unaccountable factors. In order to obtain information on a possible physiologic mechanism for asthma by reflex contraction of the bronchioles, a series of experiments was undertaken on the innervation of the mammalian bronchioles. Because the guinea pig is known to be susceptible to anaphylactic shock, as evidenced by bronchospasm, we have concentrated our investigations on this species.

METHODS. There are several methods for measuring the caliber of the bronchioles in the living animal. Dixon and Brodie, in 1903, and Swanson, in 1929, reviewed these methods. Direct plethysmography of the lung of the guinea pig was considered unwise in our experiments because of the attendant difficulties, and also because we wished to avoid extensive manipulation of the lung. The method adopted, with modifications by us for recording bronchial movement, was a device originally reported by Jackson, in 1912, and adapted for the guinea pig, by Koessler and Lewis, in 1927. Without our modification, this method consists in abolishing the natural respiratory rhythm of the animal by pithing it and by inflating the lungs with uniform rhythmic insufflations of air or oxygen. The excursions of the lungs are measured by a trocar in the pleural cavity which communicates with a sensitive tambour writing on a smoked drum.

Although there can be no question that the general employment of this method is desirable in detecting mild degrees of anaphylactic shock in guinea pigs, it is obvious that, for a study of pulmonary reflexes, the procedure of pithing the animal cannot be employed. In our studies operative procedures were carried out under local anesthesia and the animal's respirations were abolished by curare. We found it advisable, also, to measure changes in pulmonary volume by a sensitive piston recorder; even the most delicate tambour possessed an undesirable amount of limitation of excursion, and rigidity, so that the slight changes in pulmonary volume were not registered.

The technic employed by us for recording changes in pulmonary volume was, accordingly, as follows: Two-tenths cubic centimeter of 4 per cent solution of curare was injected in a vein of the foreleg of a moderately large guinea pig, thus immediately immobilizing the animal. Under local anesthesia, a cannula was quickly inserted into the trachea, and connected to the apparatus for artificial respiration. This consisted of a cylinder of oxygen from which the current was regulated by a needle valve. The stream of oxygen at a pressure of 10 mm. of mercury was interrupted rhythmically forty-five times a minute by a rotating interrupter. As the jet was interrupted, the lung became deflated passively through a small orifice in the tracheal cannula.

The shaft of a trocar needle, which was perforated with six holes, was then introduced into the pleural cavities; care was taken to avoid the intrathoracic organs. The corresponding variations in pulmonary volume, due to positive inspiration and passive expiration, were recorded by a small, free-working piston recorder, the capacity of which was 7 cc. It should be mentioned that the preparation was kept warm by conducting the experiment on a heated table.

It soon became evident that the bronchioles of such a preparation were in a state of constant activity. The slamming of a door brought about decided changes in pulmonary excursions as did also such simple procedures as carrying on a conversation after a period of silence (fig. 1 *d*, *e*, *f*, *g*). Accordingly, it became imperative to test the validity of the assumptions implied by this method of recording changes in the bronchioles of the lungs.

The chief of these assumptions is that, although the thoracic wall of the animal moves in and out with the insufflations of oxygen, it manifests no alteration in tonus during the experiment. That such alterations in the tonus of the thoracic wall can occur even in curarized preparations cannot be summarily dismissed in view of the possible rôle of the sympathetic nervous system in muscular tonus. Moreover, there is always the possibility of incomplete curarization, with resultant invisible movements. Accordingly, this method of registering pulmonary movement was corroborated. The rubber tubing from the tracheal cannula was tapped by means of a 19-gauge hypodermic needle which was connected by a piston recorder having a capacity of 7 cc. Variations in intratracheal pressure thus obtained were recorded simultaneously above the piston recorder which registered changes in pulmonary excursion. This was the technic finally adopted in all our experiments.

On vagal or cutaneous stimulation, we observed by this method an ascending curve of the lever recording intratracheal pressure which is explained as follows (fig. 1 *a*, *b;* fig. 2 *a*, *b*): As the force of the artificial inspiration is greater than the expiratory force occasioned by the elasticity of the lung, the upstroke of the writing lever, representing inspiration, is

steeper and quicker than the downstroke. In order to keep the tracing parallel with the base line, it is necessary to balance the writing lever of the piston measuring changes in intratracheal pressure by an adjustable weight. This part of the technic requires care. Any increase in resistance to the air passing into the alveoli, as by bronchoconstriction, results in a decrease

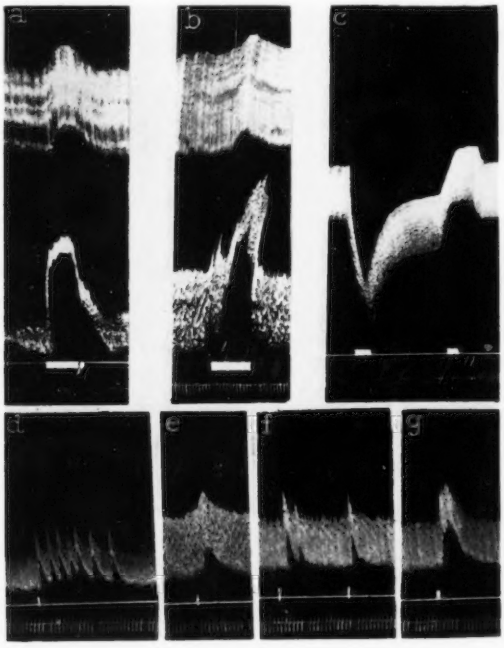

Fig. 1. Vagus nerves intact. *a*, Upper part represents intratracheal tracing, and lower, intrapleural; bronchoconstriction on vaginal stimulation. *b*, Upper part represents intratracheal tracing, and lower, intrapleural; bronchoconstriction on nasal stimulation. *c*, Intratracheal tracing; signal 1, bronchial relaxation on nasal stimulation; signal 2, bronchoconstriction on vaginal stimulation. *d*, Intrapleural tracing; bronchoconstriction produced by clapping hands near animal. *e*, Intrapleural tracing; bronchoconstriction produced by blowing on guinea pig. *f*, Intrapleural tracing; bronchoconstriction produced by tapping table. *g*, Intrapleural tracing; bronchoconstriction produced by pinching guinea pig's leg.

of the air passing to the lungs with a consequent increase in the amount offered to the tracheal piston recorder. Most of this excess escapes through the vent in the tracheal cannula, and part is registered as a longer upstroke. Hence, one observes an ascending curve in the event of bronchoconstriction (Dixon and Brodie). In the event of bronchial relaxation, the opposite is recorded (fig. 1 *c*).

Having satisfied ourselves that, by recording intratracheal pressure, we possessed an alternative means of recording changes in the lumen of the bronchioles, the following crucial experiments were performed. Tracheal pressure was recorded as usual, and under local anesthesia the thoracic cavity was thoroughly opened through the abdomen so that each pleural cavity communicated with the exterior. There now could be no question that the changes in intratracheal pressure were independent of alterations in the tonus of the thoracic muscles. The results of this technic were

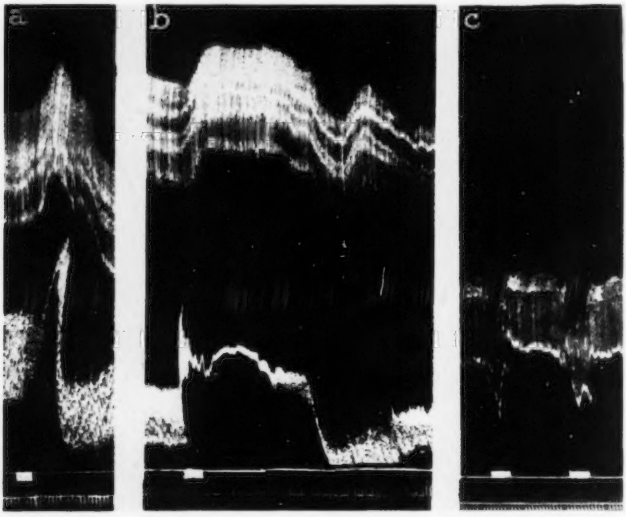

Fig. 2. *a*, Upper part represents intratracheal tracing, and lower, intrapleural; bronchoconstriction on weak faradization of right vagus nerve. *b*, Upper part represents intratracheal tracing and lower, intrapleural; bronchoconstriction on strong faradization of right vagus nerve. *c*, Intrapleural tracing; bronchial relaxation at signal 1 (moderate faradization of right vagus nerve) and at signal 2 (moderate faradization of left vagus nerve).

identical with those obtained when the thorax was closed (fig. 3 *d*). Faradic stimulation of the vaginal orifice or of the nares caused a rise in intratracheal pressure, indicating bronchoconstriction (fig. 1 *a*, *b*). Faradic stimulation of the right vagus trunk brought about the same result, but to a more marked degree (fig. 2 *a*, *b*).

There remains to consider now the possible part played by circulatory changes of sufficient magnitude so that these, and not changes in the bronchioles, would account for the results. Actually, the changes in blood pressure accompanying various pulmonary reflexes were found to be insig-

nificant (fig. 4). Swanson has reviewed the literature on this point and has shown that repeated clamping of the aorta and vena cava did not produce alteration in the bronchioles of the dog. This worker, like ourselves, measured bronchial movements by recording changes in pulmonary excursion through an intrapleural trocar. That vascular engorgement

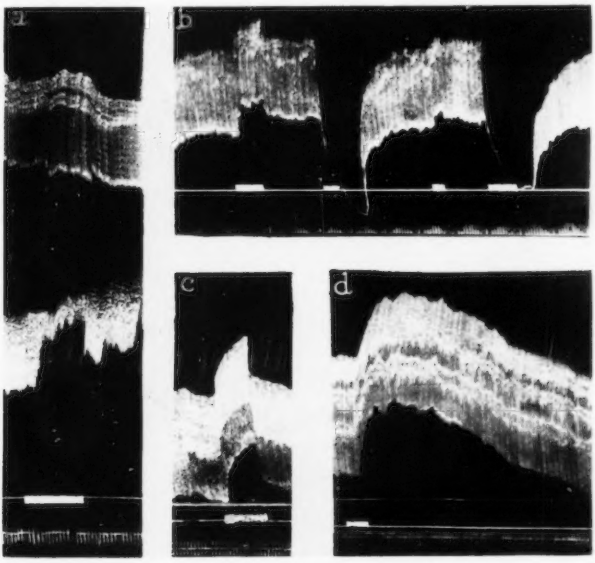

Fig. 3. *a*, Mechanical lung; upper part represents tracheal tracing, and lowes, intrapleural; signal, clamping tracheal tube, mechanically representing bronchospasm. *b*, Intrapleural tracing; signal 1, vaginal stimulation; signal 2, clamping trachea; signal 3, vaginal stimulation; signal 4, clamping trachea and vaginal stimulation (if curve at signal 1 were due to vascular causes, there should be a rise in curve on vaginal stimulation with trachea clamped at signal 4: if due to changes in tonus in thoracic wall, there should be a rise on vaginal stimulation with trachea clamped at signal 4). *c*, Mechanical lung; rubber balloon tied over end of tracheal tube and half of the balloon clamped off; intratracheal tracing; rise in intratracheal pressure resulting from less air being admitted into lung. *d*, Intratracheal tracing; vagus nerves cut and thorax opened; bronchoconstriction on vaginal stimulation.

was not responsible for the changes reported by us was shown by clamping the trachea and utilizing various effective types of stimulation; there was no rise in pulmonary volume (fig. 3 *b*).

In order to visualize the changes recorded by the foregoing technic, we constructed an artificial respiratory scheme in which the lungs were represented by a rubber balloon, and the pleural cavity by a flask with a side-

arm. The rubber balloon was placed in the flask and tied to a glass tube that perforated a tightly fitting rubber stopper. The flask was incompletely filled with water so as to make the relative size of the lungs and the pleural cavities more nearly natural. The tubes leading to the balloon and to the side of the arm of the flask were connected to corresponding piston recorders. When the amount of air admitted to the rubber balloon was curtailed by narrowing the rubber tubing with a buret clamp, the intratracheal pressure rose and the oscillations of the lever were greater. The

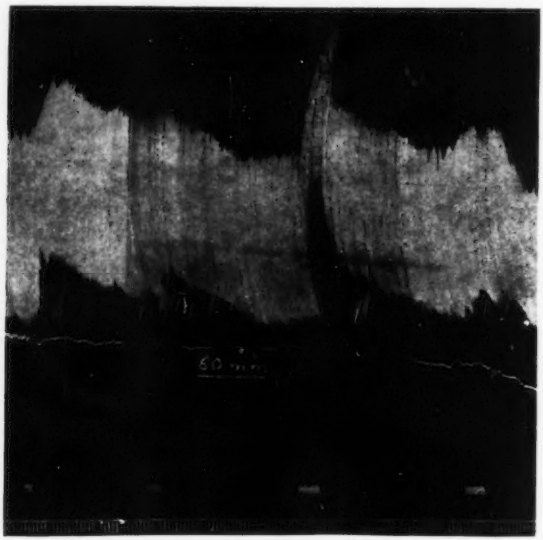

Fig. 4. Upper part represents intratracheal tracing and lower, carotid blood pressure; both vagus nerves sectioned; signal 1, pounding table, bronchoconstriction with little or no response in blood pressure; signal 2, pinching animal's leg; bronchoconstriction with some increase in blood pressure; signal 3, vaginal faradization; bronchoconstriction with some increase in blood pressure; signal 4, faradization of left vagus nerve; bronchoconstriction with fall in blood pressure.

pulmonary excursions became diminished and were recorded by shorter oscillations of the writing lever and an ascending curve denoting increased pulmonary volume (fig. 3 *a*). Complete occlusion of the trachea by the buret clamp abolished all pulmonary excursions, similar to that observed in bronchoconstriction in the living animal. Meanwhile, the amplitude of the tracheal excursions was increased.

Another mechanical experiment showing the changes observed in intratracheal tracings by a decrease in the respiratory volume is demonstrated by tying a balloon or a finger cot over the end of the tracheal cannula.

After the writing lever is properly balanced about half of the balloon is clamped off. An ascending curve is recorded, representing increased intratracheal pressure (fig. 3 *c*).

These demonstrations showed rather clearly that even marked bronchoconstriction was not followed by glaring increases in the movements of the lever. This confirmed the animal experiments and indicated that even a

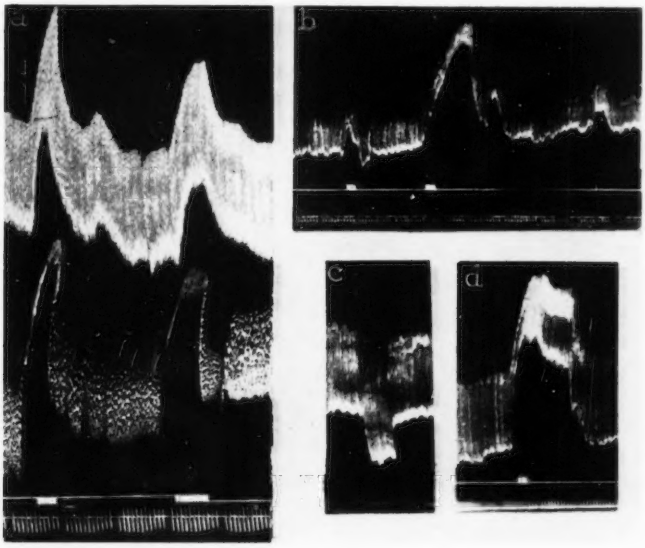

Fig. 5. Vagus nerves cut. *a*, Upper part represents intratracheal tracing and lower, intrapleural; signal 1, bronchoconstriction on nasal stimulation; signal 2, bronchoconstriction on vaginal stimulation. *b*, Intrapleural tracing showing bronchoconstriction on signal 1, pinching guinea pig's legs, at signal 2, pounding table, and at signal 3, blowing on guinea pig. *c*, Intrapleural tracing; dilatation on nasal stimulation. *d*, Intrapleural tracing; bronchoconstriction on rubbing guinea pig's hair.

slight rise in intratracheal pressure denoted considerable narrowing of the bronchioles.

Results. One of the most striking points about the behavior of a curarized guinea pig is the constant activity of the bronchioles in response to various environmental changes. It was observed early in the experiments that the pulmonary excursions were very irregular; this was puzzling until we discovered that abolishing all noises and shading the animal's eyes from the light produced an even tracing. A systematic study of the various extraneous factors influencing the animal's pulmonary volume was then

undertaken. Tapping the copper top of the table on which the animal was lying brought about great increases in the volume of the lungs. The same result was produced by slamming a door, flashing a light, carrying on a conversation after a period of quiet, and so forth (fig. 1 *d*, *e*, *f*, *g*). In general, those measures which were sudden or startling produced increased pulmonary volume. Certain types of stimuli were almost specific in the regularity with which they evoked a response, such as mild faradic stimulation of the vaginal orifice (fig. 1 *a*). In one case, creating a draught, by opening

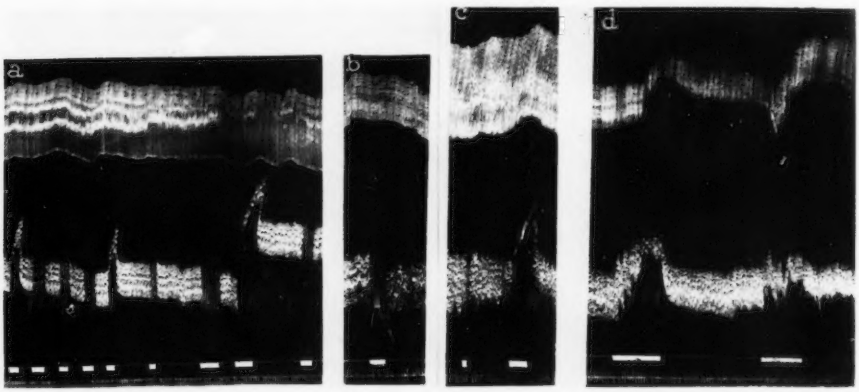

Fig. 6. Vagus nerves cut; atropine in supraparalyzing doses; in *a*, *b*, and *c* the upper part represents intratracheal tracing, and the lower, intrapleural. *a*, Signal 1, bronchoconstriction on vaginal faradization; signal 2, stimulation of left vagus nerve, no response; signal 3, bronchoconstriction on vaginal stimulation; signal 4, bronchodilatation on nasal stimulation; signal 5, bronchoconstriction on vaginal stimulation; signal 6, stimulation of right vagus nerve, no response; signal 7, bronchial relaxation on nasal stimulation; signal 8, bronchoconstriction on vaginal stimulation; signal 9, both vagus nerves stimulated, no response. *b*, Bronchial relaxation on strong faradization of right vagus nerve. *c*, Signal 1, vagus stimulation, no response; signal 2, bronchoconstriction on vaginal stimulation. *d*, Signal 1, bronchoconstriction on vaginal faradization; signal 2, bronchodilatation followed by constriction on nasal stimulation.

a window, produced bronchoconstriction. Often, blowing vigorously with the mouth in the direction of the animal produced the same result (fig. 1 *e*).

As is well known in consequence of the work of Dixon and Brodie, Carlson and Luckhardt, Mount, and others, the vagus nerves contain mostly bronchoconstrictor and some dilator fibers. It is of extreme interest, therefore, to note that sectioning the vagi in the neck or injecting enough atropine to produce the same effect, or both, do not alter the foregoing responses of the animal to these types of stimuli (fig. 5 *a*, *b*, *d*). It was this observation that made us hesitant about accepting these methods of

recording pulmonary volume, but since repetition of the experiments in preparations with an open thorax has yielded identical results, we are forced to the belief that bronchoconstrictor and bronchodilator fibers are not exclusively carried by the vagus nerves. The thoracicolumbar outflow is the probable source of these fibers and this point is now under investigation.

Certain other facts confirming the conclusions of previous workers have emerged from our experiments. Stimulation of the nose is a powerful source for the production of pulmonary reflexes. Faradic stimulation of the nasal mucous membrane always produced either bronchoconstriction or, less commonly, the reverse effect of bronchodilatation (fig. 1 *b, c*). These results, like the others we have described, were not abolished by sectioning the vagi in the neck or giving paralyzing doses of atropine, or both (fig. 5 *a, c*; fig. 6 *d*). Carlson and Luckhardt, and Dixon and Brodie, working on animals with intact vagus nerves, have commented on the great certainty with which pulmonary reflexes are produced by stimulating the nasal mucous membrane. However, the nose is not alone the source of such reflexes. A nocuous stimulus applied to the skin, even with the vagus nerves cut, may produce the same result.

That the vagus nerve contains bronchoconstrictor and bronchodilator fibers, as is stated in the literature, was frequently demonstrated during our experiments. Faradic excitation of the peripheral end of the cut vagus nerve in the neck nearly always produced marked bronchospasm (fig. 2 *a, b*). Occasionally, relaxation was recorded (fig. 2 *c*). Repetition of this experiment, after incomplete atropinization of the animal, has given bronchodilatation or no response, thus confirming Dixon and Brodie's observation that the vagus nerve possesses two types of bronchomotor fibers (fig. 6 *a, b, c*).

Comment. In this connection we would recall the work of Hargis and Mann, Reid, Mattson, Corbeille, and Barcroft and his school to the effect that the splanchnic viscera markedly change in volume as a consequence of various types of external stimuli. In general, these stimuli were of such a nature as to evoke some sort of emotional response from the animal. In the classical experiments of Hargis and Mann it was found that clapping the hands or slamming the door produced marked shrinkage of the spleen. Their experiments were conducted on relatively intact preparations, in which this organ had previously been encased aseptically in a collodion plethysmograph. The experiments reported by us likewise have been performed on relatively intact preparations, and we made the unexpected observation that such changes are neither confined to the splanchnic viscera nor are they exclusively vasomotor in nature. The contraction of the bronchioles of the guinea pig in response to various emotional stimuli amplifies the observations of Hargis and Mann and suggests the possibility that such changes are universally distributed over the entire involuntary nervous system.

The fact that bronchoconstriction can be induced in guinea pigs by various types of stimuli may have a practical bearing in certain clinical cases in which asthma appears to be definitely of psychic origin. The whole question of the innervation of the bronchioles may be considered as reopened, since there can be no reasonable doubt that reflex bronchoconstriction is recorded in curarized guinea pigs, even though bilateral vagotomy, atropinization, or both, have been done.

From time to time claims have been made by competent clinical observers that certain types of asthma are reflex in origin and that the correction of various pathologic conditions in the nose or elsewhere has been followed by cure of the asthma. Such observations have found little support in experimental observations on animals. One reason for this is, undoubtedly, that the employment of a general anesthetic has effectively abolished the reflexes. When one considers that a patient under the influence of a drug, such as morphine or ether, is relieved of his asthma, it is not remarkable that an animal under a general anesthetic should not readily manifest reflex bronchospasm.

SUMMARY

A study of pulmonary reflexes was undertaken in curarized, locally anesthetized guinea pigs. The caliber of the bronchioles was measured by recording the movements of the lung through an intrapleural trocar, and by measuring variations in intratracheal pressure.

It was found, in confirmation of the work of others, that the vagus nerve possessed both bronchoconstrictor and bronchodilator fibers. In addition, the observation was made that the bronchioles of a guinea pig were extraordinarily sensitive to such stimuli as clapping the hands, or stimulating the nose or the skin. Sectioning the vagi in the neck, paralyzing doses of atropine, or both, did not abolish these reflexes. Since the parasympathetic nerves had been eliminated, we must conclude that both dilator and constrictor reflexes are carried by the sympathetic nerves to the bronchial muscles.

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EPINEPHRIN ACTION IN RELATION TO THE HYDROSTATIC PRESSURE EFFECT ON THE CONTRACTION OF CARDIAC MUSCLE

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The influence of epinephrin on the contraction of cardiac muscle is of interest in that it produces a stimulating action which is perhaps greater than that of any other known chemical substance. On the other hand, more striking effects of the same general nature may be obtained by employing, as we have done, the purely physical agent, hydrostatic pressure (1928). These considerations suggested a study of the action on heart muscle of these dissimilar agencies when employed simultaneously. Will their effects be produced independently of each other or will there be some modification of the pressure effect due to the presence of the epinephrin, or vice versa? To what extent may the total augmentation of activity be raised?

An answer to these questions, which has been attempted in the present investigation, should be of value in formulating an hypothesis for the explanation of the action of pressure on muscular contraction.

METHOD. The observations have all been made on the isolated auricle or ventricle of the terrapin heart, which was so arranged that the tension of the isometric contractions were optically recorded from a torsion wire lever. The tissue was kept immersed in a chamber containing Ringer's solution to which pressure was applied or epinephrin added as required. The apparatus and methods of applying pressure were the same as those previously described (1928; 1930c). The tension data were obtained from photographs of the response of non-rhythmic preparations to single break induction shocks. Usually the preparation was stimulated regularly at 1 minute intervals.

In carrying out an experiment the general procedure has been to take a series of control photographs for the purpose of establishing the tension values for the preparation. These were used as a standard of comparison for all subsequent measurements made under altered physiological conditions. Secondly, the pressure was raised to between 1200 and 1600 pounds per square inch and a further series of records made. The pressure

was then released and epinephrin added to the chamber in an amount giving a concentration of 1:250,000, following which a third series of contractions was recorded. Lastly, the pressure was again raised to the value previously employed and a final series of photographs made for the purpose of showing the tension developed under the combined influence of pressure and epinephrin.

RESULTS. The data from 16 experiments on auricular muscle and 13 experiments on ventricular muscle are summarized in tables 1 and 2.

TABLE 1

The influence of pressure on the tension developed by the ventricular muscle of the turtle in relation to epinephrin stimulation

EXPERIMENT NUMBER	PRESSURE	INCREASE IN TENSION			
		Pressure	Epinephrin	Pressure + epinephrin	Excess
	pounds	*per cent*	*per cent*	*per cent*	*per cent*
102	1,200	40.7	11.3	50.7	−1.3
103	1,200	70.8	54.2	154.2	+29.2
104	1,200	50.0	48.1	67.2	−30.9
106	1,200	83.3	50.0	161.1	+27.8
107	1,200	67.5	47.8	116.7	+1.4
108	1,200	102.7	102.7	181.1	−24.3
109	1,200	68.7	15.4	97.3	+12.2
110	1,200	84.2	31.6	115.9	+0.1
111	1,200	90.0	66.6	173.3	+16.7
112	1,600	100.7	40.3	193.6	+47.4
113	1,600	183.2	80.9	327.5	+63.4
114	1,600	55.0	16.9	66.2	−5.7
116	1,600	45.1	14.3	47.2	−12.2
117	1,600	40.9	−2.2	40.9	+2.2
118	1,575	66.2	16.0	91.5	+13.7
119	1,500	50.0	1.6	58.3	+16.6
Average....	1,367	74.9	37.2	121.4	+9 7
		112.1			

The pressures employed are shown for each experiment in the second column in both tables. Under the general heading of "increase in tension" are four columns showing the stimulation observed as a result of the various procedures, the figure in every case being expressed as a percentage of the initial (control) value. The first column under this heading gives the percentage increase in tension resulting from the application of pressure alone, and the third for the combined influence of pressure and epinephrin. The last column, designated as "excess," gives the excess (or deficiency) of the observed values of the tension for the combined action of pressure

and epinephrin over the algebraic sum of the same quantities observed separately, as recorded in the first two columns. Photographs showing the result from a typical experiment are reproduced in figure 1.

If epinephrin and pressure exert their influence upon two entirely separate parts of the mechanism for contraction, then the tension developed under the combined action might be expected to equal the algebraic sum of the stimulation produced by the two agents separately. Actually this is approximately true, as shown in the experimental data in the tables, and it is quite evident, therefore, that the mechanism of action of one agent is not such as to interfere with that of the other. On the contrary, in a

TABLE 2

The influence of pressure on the tension developed by the auricular muscle of the turtle in relation to epinephrin stimulation

EXPERIMENT NUMBER	PRESSURE	INCREASE IN TENSION			
		Pressure	Epinephrin	Pressure + epinephrin	Excess
	pounds	*per cent*	*per cent*	*per cent*	*per cent*
105	1,200	19 8	−24 5	4 2	+8 9
106	1,200	34 9	19 1	68 9	+14 9
107	1,200	30 0	12 8	50 4	+7 6
109	1,200	36 0	−20 0	24 0	+8 0
110	1,200	47 3	16 4	47 3	−16 4
111	1,200	60 8	74 3	150 0	+14 9
112	1,600	84 6	29 2	134 6	+20 8
113	1,600	141 2	1 4	153 1	+10 5
114	1,600	35 0	−5 0	55 0	+25 0
115	1,600	25 3	1 7	40 5	+13 5
117	1,600	45 8	29 1	62 5	−12 4
118	1,550	61 5	7 7	88 4	+19 2
119	1,500	27 0	17 0	42 3	−1 7
Average....	1,404	49 9	12 2	70 9	+8 7
		62 1			

majority of the experiments for both the auricle and ventricle a real superiority is shown for the development of tension under the influence of the simultaneous presence of epinephrin and pressure, as compared to the sum of the two influences acting separately but in succession. For the entire series of 29 experiments the improvement averages about 9 per cent, and this includes the 8 experiments which are negative in this respect. These results are strongly suggestive of a synergistic influence between the two agents under study—hydrostatic pressure and epinephrin.

Discussion. The data given in the two tables are significant, we believe, in showing an actual increase in the effectiveness of pressure in the

presence of epinephrin. It should be noted that this conclusion is based on the percentage increase in tension as compared with the control values obtained at the start of the experiment before any experimental procedure had been applied. In other words, in absolute units the tension increase produced by a given change in pressure is greater in a majority of the experiments after the application of the epinephrin. However, if one takes the augmentation in tension resulting from pressure in the "absence of" and in the "presence of" epinephrin (fig. 1, *B* and *D*) and calculates the percentage increase from the immediately preceding pre-pressure value (fig. 1, *A* and *C*), then the result obtained usually shows an increase actually less in the presence of epinephrin. The latter method of evaluating the results is not acceptable because it obscures the greater increase in grams of tension that has been observed to occur under the influence of a

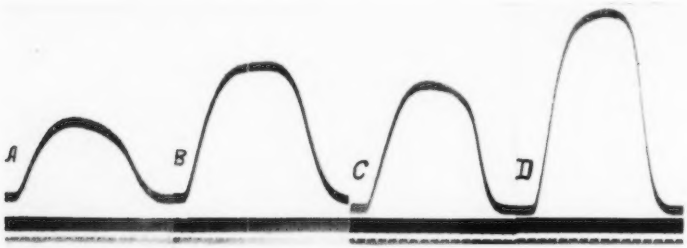

Fig. 1. Curves of the tension developed in response to single induction shocks by a ventricle preparation of the terrapin heart. *A*, control; *B*, a "pressure effect" (1200 lbs. compression) of 74.4 per cent; *C*, an "epinephrin effect" (1:250,000 concentration), amounting to 61.7 per cent above the control; and *D*, a "pressure effect" superimposed on the "epinephrin effect," amounting to 158.7 per cent above the control. Time line signifies $\frac{1}{5}$ second intervals.

given pressure when epinephrin is present and, moreover, the correct standard of comparison would appear to be the tension developed by the muscle under as nearly natural conditions as possible.

In this series of experiments, as in those previously published, there is a wide variation in the stimulation produced by the pressure. They are not due to a chance experimental error, but are significant of a difference in the physiological condition of the tissue, possibly due in part to the treatment given it after removal from the animal (1930b, c). There is, however, a remarkable constancy in the responsiveness of a given preparation to the action of hydrostatic pressure, and it is of interest to note that a certain degree of correlation is shown in the stimulation produced in the auricle and ventricle taken from the same animal, as may be seen by comparing tables 1 and 2, the results bearing identical numbers which signify preparations obtained from the same heart.

The variability in the effect of epinephrin is even greater than that of pressure, for in certain instances no stimulation whatever has been observed, and occasionally there has been a falling off in the tension developed. It is significant perhaps that even in the absence of a direct stimulating action by epinephrin its influence in increasing the action of pressure on the tension has been demonstrated in every instance.

We have made no allowance for the gradual decrease in the tension developed by the muscle which inevitably occurs as the experiment proceeds. This is associated with a decline in the general functional condition so that there is an increased slackness of the muscle that undoubtedly accounts for the lessening of tension. This decrease has been recorded always in control experiments, and in the present series it would give a lower value to the records made later and would tend to obscure the evidence of a synergistic action, since the observations on the combined action of epinephrin and pressure of necessity were made at the terminal part of the experiment. This consideration suggests that the average figure of about 9 per cent for the increased effectiveness of pressure when combined with epinephrin is less than the true value.

The increase in tension under the combined influence of pressure and epinephrin, averaging 70.9 per cent for the auricles and 121.4 per cent for the ventricles, indicates a remarkable stimulation of the contractile process, but it does not represent the maximum of stimulation possible, for recent experiments with an apparatus made to withstand much higher pressures have shown an augmentation of over 400 per cent in the contractile power of the muscle. Such an extreme stepping-up of the capacity of heart muscle to develop tension as occurs in these instances appears, however, to be close to the upper limit of contraction of the muscular machine, since results with preparations augmented by these high pressures show a response no higher when the muscle is treated previously with epinephrin.

SUMMARY

The stimulating action of increased hydrostatic pressure on the tension developed by cardiac muscle is augmented by the presence of epinephrin. This augmentation is greater than the stimulation produced by epinephrin alone, indicating a synergistic action between these two agents.

Through the combined action of pressure (1200 to 1600 pounds) and epinephrin (1:250,000) the average value for the tension developed was increased 70.9 per cent in the case of the auricles and 121.4 per cent in the case of the ventricles.

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COMPARATIVE TOXICITY OF BLOOD AFTER ARTERIOTOMY, ASPHYXIATION AND INJURIES TO THE BRAIN

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The present writer has described in numerous publications a phytopharmacological method of studying the toxicity of normal and pathological blood specimens. He has pointed out that toxins and various metabolic products elaborated by animals and found in the blood are much more poisonous for living plant protoplasm and living plant tissues than for biological test objects derived from animals. In this way, the toxicity for the growth of *Lupinus albus* seedlings was determined in case of normal blood serum, on the one hand, and the more toxic effect produced on seedlings by blood sera obtained from certain pathological conditions, on the other (1). In view of the great sensitiveness of living seedlings for toxic constituents of the whole blood and blood sera, it was deemed worth while to investigate the pharmacological and toxicological properties of the blood obtained from various animals, which were killed under different conditions. The author was especially desirous of inquiring into the relative pharmacological and toxicological properties of freshly shed arterial blood, on the one hand, and blood specimens obtained from animals of the same species which had been killed by asphyxiation, or by decerebration or other injury to the brain, on the other. The results of such an investigation were very interesting and clear-cut and are reported in this place.

MATERIAL STUDIED. The blood serum or the whole blood, as the case might be, was studied from the following kinds of animals: the white mouse, the white rat, the guinea pig, the rabbit, the cat, the dog, the domestic pigeon and the domestic hen. In case of mice, blood was obtained by direct puncture of the heart with a fine syringe and hypodermic needle and compared with blood obtained in the same way after crushing the brain by a blow on the head. The amount of blood obtained from mice was so small that solutions of the whole blood dissolved in plant physiological saline were used in case of this animal. In every other instance, the experiments were performed with blood serum instead of the whole blood because this could be more accurately measured.

Blood specimens from rats and guinea pigs were obtained for comparative purposes in two ways: by severing the blood vessels of the throat with a sharp knife or scissors, on the one hand, and after paralyzing the brain of the animal with a blow on the head, on the other. When the animals were killed by contusion of the brain, about five minutes were allowed to elapse before obtaining the blood by drawing it directly from the heart or from the large vessels.

Blood specimens for comparative purposes from the pigeon and hen were also obtained in two ways. Freshly shed blood was obtained by severing the vessels of the neck, and this was compared with blood obtained several minutes after breaking the neck of another bird of the same species.

Blood specimens from the rabbit, cat, and dog were obtained in a different way. Fresh arterial blood was obtained by tapping the carotid arteries after cannulating them. Asphyxial blood was obtained by pushing ether anesthesia until all the respiratory movements ceased and waiting until the heart-beat became very feeble. When that stage was reached, blood was drawn off from the carotid artery. Still other experiments were made by pithing the brain of the animals and maintaining artificial respiration and drawing the blood from the carotid artery after waiting a few minutes. Finally, another specimen of blood was obtained from such decerebrated animals after suspending artificial respiration. The samples of blood obtained by these various methods were then compared pharmacologically. Specimens of blood obtained from rabbits, cats and dogs were procured in some experiments from the same animals: first by drawing off a specimen of arterial blood under light ether anesthesia; secondly, by producing asphyxiation or cessation of the respiration with excessive doses of ether; thirdly, after decerebrating the animal and maintaining an artificial respiration; and, fourthly, after decerebrating the animal and discontinuing the artificial respiration. In other experiments, the various samples were obtained from different individuals of the same species, using a fresh animal for each method. In all the experiments performed, care was taken to maintain deep anesthesia, and none of the animals was allowed to revive after the operations but was killed with ether.

METHOD OF STUDY. The blood sera were separated by centrifugalizing the blood specimens and the phytopharmacological or phytotoxic properties of the various samples were studied on living seedlings of *Lupinus albus* in the following manner. According to the methods described in detail elsewhere (2), the seedlings were germinated from seeds which had been soaked in water for twenty-four hours, and then planted in moist finely ground sphagnum moss. Specimens with roots measuring from 25 to 45 mm. were selected. These were carefully washed in water and placed in upright test tubes of hard glass containing equal parts of distilled water and a plant physiological nutrient solution. The solution

used was the so-called Shive solution containing definite proportions of diacid potassium phosphate, calcium nitrate and magnesium sulphate (3). Eight or ten seedlings were carefully measured as to the length of roots and placed in such a solution for controls. In a similar manner other rows of hard-glass tubes were filled with equal parts of distilled water and Shive solution, containing, in addition, one per cent of blood serum. In these solutions there were placed other carefully measured seedlings from the same crop, and the whole outfit was then put away in a dark place and kept at a temperature of from 20° to 21°C. for twenty-four hours. On the following day the increment in length of the roots of each seedling in the different rows was carefully measured with a millimeter rule, and the amount of growth in the various blood sera solutions was compared with the growth of the control seedlings in the plant physiological solution, consisting of equal parts of distilled water and Shive. This ratio is called by the author the *phytotoxic index*.

Results. In the subjoined table are exhibited the kind of blood serum examined, species of animal, and the phytotoxic index, or the degree of growth of the roots as compared with the normal controls and expressed in form of percentages. In the case of the mouse, whole blood was dissolved in the physiological solutions. In case of the other animals, the blood serum was utilized. The indices shown in the table are the average of a large number of experiments and it should be borne in mind that, in each experiment, the phytotoxic index is obtained from an average of 8 to 10 individual seedlings for each solution used. This wealth of test objects is one great advantage in connection with plant physiological methods as it is practically impossible to perform eight or ten animal experiments with each specimen in one day and, still less, with a dozen or more different specimens of blood. After an experience of six years the author has found that when seedlings are carefully chosen and accurately measured, variations in the readings of a series of plants in the hands of an experienced experimenter are usually not more than 5 per cent, that is, when repeated tests are made with the same sample of blood serum under uniform conditions of experimentation, a variation of not more than 5 per cent is usually noted in the readings. Bearing this in mind, it will be seen that in all the animals studied there was a definite difference in the phytotoxic properties between freshly drawn blood and blood obtained under other conditions. Small differences were noted in the fowl, namely, the pigeon and the hen. Very marked differences were noted in the mouse, rat, guinea pig, rabbit, cat and dog. Furthermore, it is of interest to note the differences obtained with the large animals after asphyxia, on the one hand, and decerebration, or other injury to the brain, on the other, were very striking indeed.

Although ether anesthesia was employed in the various experiments, the rôle of ether on the phytotoxic properties of the blood specimens can be

discounted as negligible because all of the ether was volatilized when preparing the solutions and many of the experiments were performed repeatedly on specimens of blood and blood serum which stood in the ice chest or in the room for several days so that no trace of ether could be detected by sense of smell or otherwise.

Discussion. The greater toxicity of blood after injury to the head, and more particularly after decerebration or pithing of the brain, is prob-

TABLE 1

ANIMAL	KIND OF BLOOD SERA	PHYTOTOXIC INDEX
		per cent
White mouse	Freshly drawn	63
	After decerebration	33
Rat (Mus norvegicus)	Freshly shed	66
	After blow on head	45
Guinea pig	Freshly shed	69
	After blow on head	54
Pigeon	Freshly shed	67
	After breaking neck	58
Hen	Freshly shed	57
	After decerebration	50
Rabbit	Freshly drawn arterial	77
	In extreme asphyxia	66
	Decerebrated	51
Cat	Freshly drawn arterial	70
	Extreme asphyxia	56
	Decerebrated but oxygenated	54
	Decerebrated and asphyxiated	50
Dog	Freshly drawn arterial	68
	Extreme asphyxia	61
	Decerebrated but oxygenated	53
	Decerebrated and asphyxiated	49

ably due to the setting free in the circulation of toxic bodies either from the brain itself or, perhaps, indirectly as a result of such injury from other organs, such as the glands of internal secretion, etc. The greater toxicity of the blood after asphyxiation as compared with freshly drawn arterial blood cannot be explained by differences in the oxygenation of the blood because after a serum has been diluted with a physiological saline the rela-

tive proportions of oxygen and carbon dioxide are practically equalized and cannot play a rôle as far as the growth of the plants is concerned under the conditions of the experiment. This, of course, would have been very different in case of asphyxia produced by such a poison gas as carbon monoxide. In carbon monoxide poisoning, Macht, Blackman and Swigart (4) have shown that the hemoglobin compound formed of carbon-monoxide hemoglobin is of a stable nature and even when dissolved in plant-physiological solutions is much more toxic than ordinary hemoglobin. The results obtained in the present investigation therefore are of considerable interest from a purely physiological point of view. They may also be of considerable interest from the standpoint of hygiene and more particularly in connection with methods of slaughtering cattle and other animals for future consumption.

SUMMARY

1. Comparative studies were made on the phytotoxic effects of blood specimens obtained from different animals after arteriotomy, extreme asphyxiation with ether, and injuries to the brain.

2. Blood specimens from asphyxiated and decerebrated animals of various kinds were found to be more toxic for living seedlings of *Lupinus albus* than freshly shed arterial blood from the same species of animal.

3. The difference in the toxicity between the blood obtained by arteriotomy, on the one hand, and blood obtained after asphyxiation or injury to the brain of animals, on the other, cannot be attributed to the anesthetic used nor to a difference in the oxygenation of the blood but must be regarded as due to a setting free in the blood of toxic substances produced as a result of the asphyxia or injury to the brain.

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STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE

I. Normal Motility in the Dog, Recorded by the Tandem Balloon Method

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This work was undertaken in an attempt to obtain suitable controls for a pharmacological study of the colon. At the time the study was begun, we were using single balloons, inserted through a cecostomy into the proximal or middle colon, in unanesthetized dogs. With this method we were frequently unable to detect marked alteration of activity following injection of the drugs we were studying. In some cases both the ensuing period of activity and the period of quiet were increased in duration, so that it was impossible to state whether the total activity was increased or decreased. The single balloon did not reveal direction of propagation of the contractions which it recorded, and since the quantitative estimate which it afforded of total motor activity did not lend itself easily to interpretation, it was considered advisable to study the contractions of the colon with reference to the number and direction of running waves.

For the study of character of activity, the triple tandem balloon devised by Templeton and Bollens (1) was used. The three compartments of this balloon system were each 3 cm. long, the whole system having a total length of 10 cm. The balloons were inserted through the cecostomy so that the butt balloon lay at the level of the ileo-cecal junction. The three compartments were distended with approximately equal volumes of air as measured by inflating the water manometers which were used for recording to a height of 10 cm. of water, with the balloons clamped off, and allowing this volume of air to inflate the balloons.

The triple tandem balloon was also used to study the distal colon. The balloons were inserted through the anus so that the lowest lay just within the internal anal sphincter, and distended with the same volumes of air as used in the proximal colon. The study of the distal colon was carried out on both unoperated, normal dogs, and upon dogs with cecostomies. On account of the difficulty encountered in inserting balloons in the distal colon through the hard fecal masses usually found, small enemata of

paraffin oil were usually given two hours before inserting the balloons. The insertion was further facilitated by attaching the balloons to a rod which was removed after the balloons were in place.

For the purpose of correlating proximal and distal colon activity, longer triple balloon systems were used, with a total length of 20 cm. for each system, the greater length being obtained by increasing the space between compartments to 5 cm., keeping the compartments the same size as in the shorter system. One of these systems was inserted through the cecostomy and the other through the anus, and the compartments inflated as before. The position of the six balloons was checked fluoroscopically and in acute animals, and was found to be as follows:

Balloon I—at the level of the ileo-cecal junction.
Balloon II—middle of the transverse colon.
Balloon III—at the splenic flexure.
Balloon IV—in the descending colon between the levels of the third and fourth lumbar vertebrae.
Balloon V—at the level of the lumbo-sacral articulation.
Balloon VI—just within the internal anal sphincter.

In this work no attempt was made to evacuate the colon, or any part of it, the purpose being to record the motor activity of each segment with as little alteration as possible of its contents. The procedures followed did not produce defecation in any case during the period of observation. Diet

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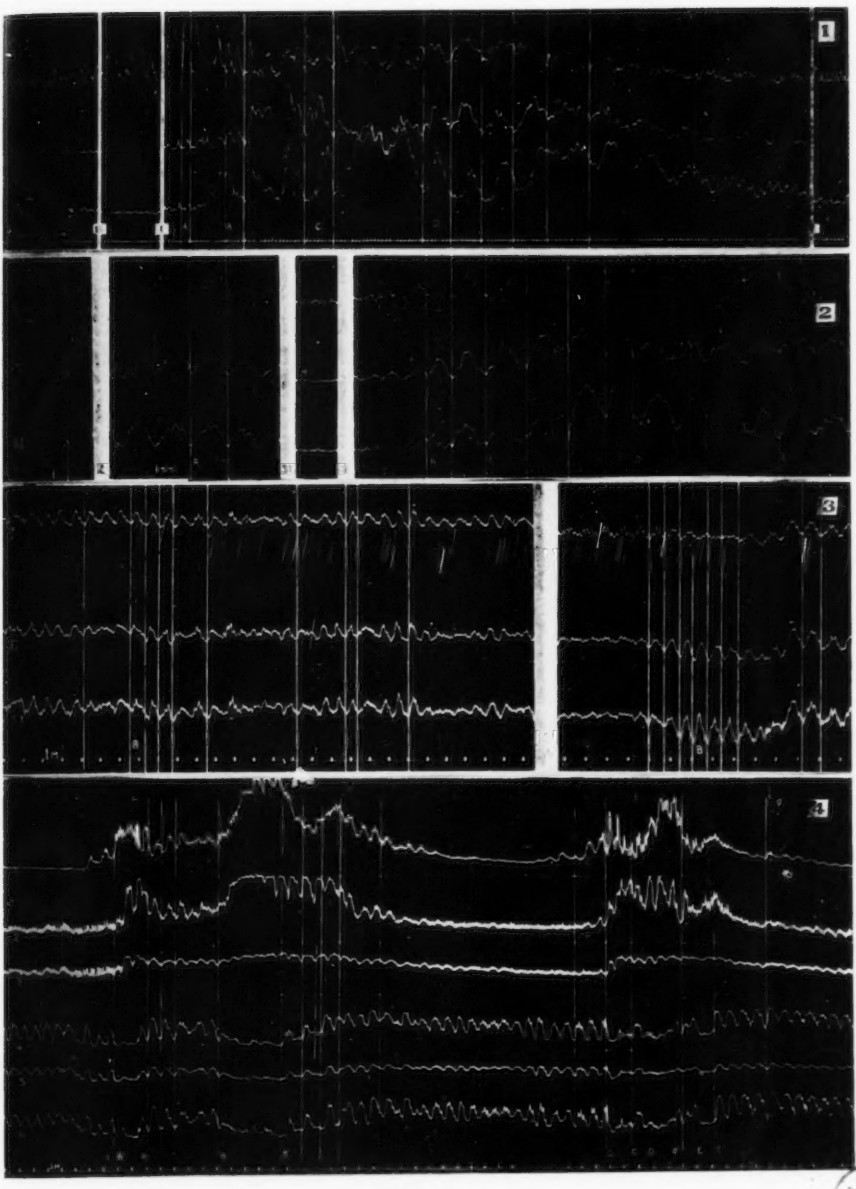
Fig. 1. Three-balloon tracing of proximal colon. Balloon I at ileocecal junction. Time interval equals five seconds. *A*, type 2 contraction on upper balloon, type 1 contraction on two lower balloons with one-half the duration of the type 2 contraction. *B*, first appearance of type 2 contractions on balloon III. *C*, delayed type 2 contractions on balloon III, with agreement of superimposed type 1 contractions on three balloons. D, period of type 2 contractions delayed on lower balloon, with agreement of superimposed type 1 contractions.

Fig. 2. Three-balloon tracing of proximal colon. Balloon I at ileocecal junction. Time interval 5 seconds. *A*, low type 2 contractions on upper balloon, high type 1 contractions. *B*, type 2 contractions delayed oralward. *C*, type 2 contractions simultaneous. *D*, type 2 contractions delayed on both upper and lower balloons.

Fig. 3. Three-balloon tracing of distal colon. Balloon III just within internal anal sphincter. Time interval 1 minute. *A*, depression in tone, with delay in type 2 contractions on upper and lower balloon. *B*, depression in tone, with delay in type 2 contractions on two upper balloons. Between *A* and *B* type 2 contractions are simultaneous.

Fig. 4. Six-balloon tracing of entire colon. Balloon I at ileocecal junction. Balloon VI just within internal anal sphincter. Distance between adjacent balloons approximately 5 cm. Time interval, 1 minute. *A*, *B*, *C*, *D* and *E*, depressions in tone and activity of distal colon, coinciding with augmented tone and activity in proximal. *A'*, *B'*, *C'*, *D'* and *E'*, return of activity in distal balloons, coinciding with depression in proximal balloons. Balloon III follows two upper balloons more closely than the distal balloons, but is not depressed by distal colon activity.



Figs. 1 to 4

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was carefully standardized, and tracings were taken 15 to 18 hours after the last feeding to insure, as nearly as possible, similarity of conditions.

In the proximal colon, at least three types of contractions were observed with the triple balloon. For purposes of discussion they are classified as follows:

1. A rapid contraction which appears on the tracings as a simple curve, except for respiratory impulses. This type will be referred to as type 1 contractions.

2. A slower contraction, surmounted by the more rapid type 1 contractions. This will be referred to as type 2.

3. Tone changes, usually surmounted by type 2 contractions.

Activity in the proximal colon was found to be distinctly periodic, the periods consisting usually of type 1 and type 2 contractions accompanied by a tone change of considerable amplitude. Preceding the tone rise, the balloon nearest the cecum frequently recorded type 1 contractions of increasing amplitude on level tone (fig. 1). During this period the second and third balloons were usually quiet. Balloon three usually began to record type 2 contractions first, followed by the second, and finally the first balloon. When type 2 contractions began to appear on balloon I a tone rise occurred, which appeared soon after on balloon II, and finally on balloon III.

During the first portion of the period, on the rise in tone, type 2 contractions which could be related by contour, duration, and position on the tone change, were usually progressively delayed in their appearance on the lower balloons. Toward the end of the period of activity, the related type 2 contractions usually began and ended simultaneously on all the balloons, or were delayed in their appearance on the balloon nearest the cecum, occurring simultaneously on the lower two (figs. 1 and 2). The type 1 contractions were recorded simultaneously on all balloons, even when occurring upon delayed type 2 contractions. It has never been possible, with this technique, to record delay in appearance of related type 1 contractions on the several balloons, although frequently the type 1 contractions appearing on one balloon could not be found on the other balloons (fig. 1). This was particularly true in the case of delayed type 2 contractions. The delay was found to be equal in duration to one, or an integral multiple of the type 1 contractions. At times, no type 1 contractions appeared during the delay in appearance of type 2 contractions; at times type 1 contractions appeared in the interval between adjacent type 2 contractions (fig. 2, D et seq.).

Between periods of activity in the proximal colon intervals of almost complete quiet were recorded (fig. 2).

In the distal colon, three-balloon tracings recorded activity classifiable as in the proximal. Tone changes were less pronounced, however, the principal changes in tone being sudden depressions with a gradual return to the former level, or slightly above. Type 1 and type 2 contractions were

of lower amplitude than in the proximal colon, and much more regular in height and rate. Delayed type 2 contractions were less frequent than in the proximal; the delayed contractions, when they did appear, usually appeared on the depressions in tone, and on the return. On the depression in tone, there was a tendency toward delay oralward, on the rise to former level or above, toward delay analward. However, the direction of delay was frequently reversed, and even more frequently, the simultaneity of occurrence of the type 2 contractions on all three balloons was uninterrupted during the tone change. In the intervals between tone changes, the distal colon was not quiet. Type 2 contractions, agreeing in phase on all the balloons, continued until the next following tone change (fig. 3).

It was noted on six-balloon tracings that activity of the type found in the proximal colon, with periods of activity surmounting high tone changes, and separated by intervals of nearly complete quiet, extended as far as the splenic flexure. In this region there was a rather sudden alteration of activity to that found in the distal colon with the three-balloon system (fig. 4). The sharp depressions of tone noted in the distal colon were found to occur coincidently with rises in tone and activity in the proximal. The augmentation of tone and activity in the distal colon following the depression was seen to occur at the time of diminution in the tone and activity of the proximal colon (fig. 4).

The anally-delayed contractions of the proximal colon were frequently traced as far as the fourth balloon (fig. 5), less frequently to the fifth (fig. 6 *K*) and only occasionally to the sixth (fig. 6 *F*). When anally-delayed contractions made their appearance in the proximal colon, there was depression of tone and activity in the distal colon. When these contractions, appearing on progressively lower balloons, finally appeared in the distal colon, there was alteration in direction of delay in the proximal colon, either to simultaneous contractions, or to contractions delayed orally, usually associated with diminution in tone (fig. 6). This alteration of proximal colon activity most frequently occurred at the time when anally-delayed contractions appeared on the fourth balloon (fig. 5, 1st period, fig. 6). The fifth and sixth balloons usually responded simultaneously with the fourth. However, less frequently the alteration of proximal colon activity occurred with the appearance of anally-delayed contractions on balloon V, the sixth responding simultaneously with the fifth; and still more rarely, the alteration occurred with appearance of anally-delayed contractions on balloon VI (fig. 6, *L*).

In some tracings, the distal colon was found to be almost completely quiet, proximal colon activity extending variable distances into the distal colon, and constituting the only interruption in the inactivity of that segment (fig. 5). The appearance of this activity in the distal colon was associated with diminution in activity in the proximal. In other cases,

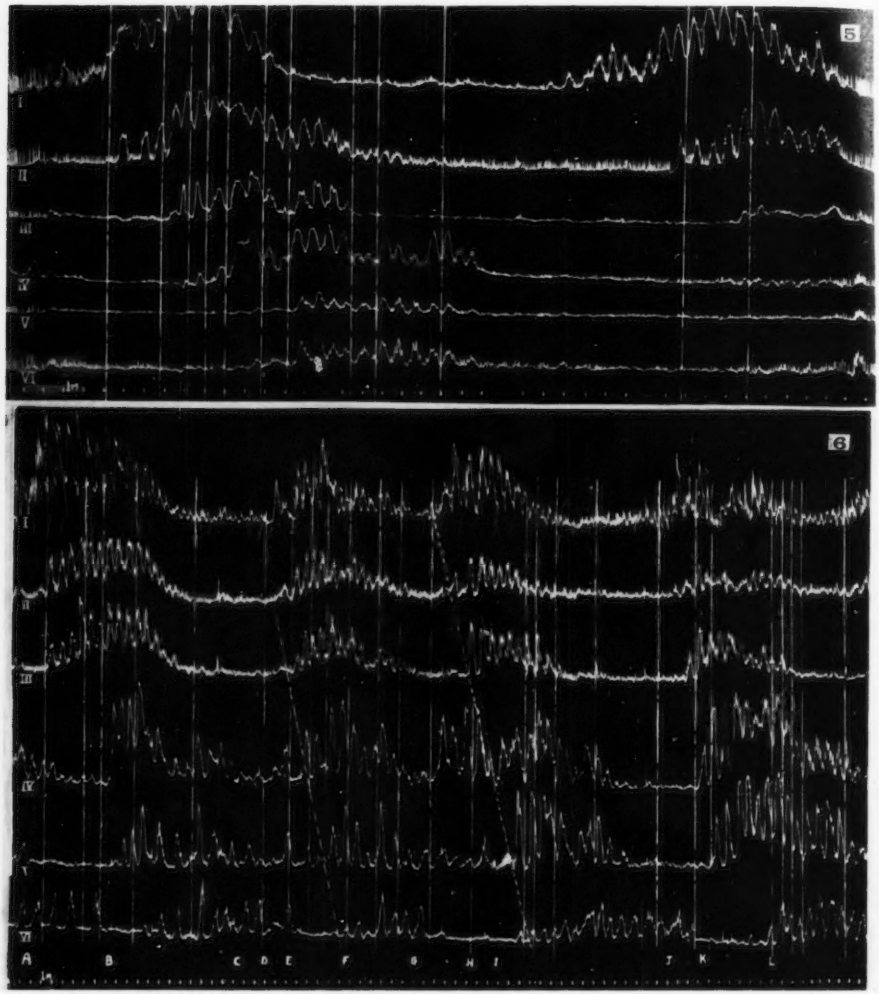

Fig. 5. Six-balloon tracing of entire colon. Balloon I at ileocecal junction. Balloon VI just within internal anal sphincter. Time interval 1 minute.

Fig. 6. Six-balloon tracing of entire colon. Balloon I at ileocecal junction, balloon VI just within internal anal sphincter. Time interval 1 minute. *A*, type 2 contractions delayed analward on balloons I and II, diminishing activity in distal balloons. *B*, sudden augmentation of balloon IV, with shift to simultaneous contractions on three upper balloons, and diminution in three upper balloons as three lower become active. *C*, alteration of anally-delayed contractions on three lower

activity of the type found to be characteristic of the distal colon was found in the proximal colon as well, long segments recording type 2 contractions which occurred rhythmically, showing agreement in phase on all balloons. In these cases, activity in the proximal colon was not found to have any appreciable effect on that of the distal, or distal colon activity on that of the proximal.

Bayliss and Starling (2) described the motor activity of the dog's colon as peristaltic, peristaltic waves passing from the ileum onto the colon and travelling as far as the middle colon. Elliott and Smith (3) confirmed this observation, in general, and also described a progressive massive contraction which was not followed by relaxation, but which spread from the ileocecal junction to the anal end, and left the entire colon a rigid, narrow tube. Being unable to find antiperistalsis in the dog's proximal colon by their method of direct observation they concluded that the dog had no true physiological proximal colon. Their work on the cat, rat, guinea pig, and other mammals, indicates that antiperistalsis is the usual finding in the proximal colon, near the cecum. The observations of Cannon (4), Zondek (5), Lurje (6), and many others on the same animals is in agreement. Work on the colon of the dog, however, has usually failed to reveal antiperistalsis near the cecum. Thomas and Kuntz (7) observed antiperistalsis near the cecum in one dog, in a series of experiments in which massive doses of nicotine were given to anesthetized animals.

Our work indicates that the mechanism of progressive delay in contractions is operative in both an aboral and an oral direction in the dog's colon. Some tracings show modifications of the simultaneous contractions so that the picture of running waves is presented. We have hesitated in classifying these as peristaltic and antiperistaltic waves because of the fact that, although they seem grossly to be related, there are local differences in the response of the different segments to such related waves, so as to suggest that they are more local in character than the use of these terms might imply. Currie and Henderson (8) observed two types of contractions in the guinea pig's colon, and thought that the larger contractions seemed to be produced by summation of the smaller. The appearance of delayed type

balloons to simultaneous contractions. *D-E*, appearance of anally-delayed contractions in upper balloons with depression in lower. The broken line connects points of beginning of anally-delayed contractions on the various balloons. *F*, appearance of anally-delayed contractions on balloon VI, with depression in upper set. *G*, beginning activity in balloons I and IV. Complete depression of balloon VI. *H*, contractions in three upper balloons show no relationship to those in lower. *I*, depression of balloon IV as activity of proximal colon reaches that segment. *J*, slight diminution in balloon VI with beginning activity on balloon I. *K*, complete quiescence in balloon VI as activity begins on IV. Contractions are delayed on balloon I. *L*, recovery in balloon VI, with depression in all balloons above.

2 contractions, with agreement in phase of the superimposed type 1 contractions, may argue for a complete separation of the mechanisms producing the two types. On the other hand, the fact that the larger contractions frequently follow a period of the smaller, increase in amplitude as the smaller contractions decrease, and that even in the case of the strongest contractions, the smaller curves can usually be seen superimposed, might indicate production of the larger contractions from the smaller. In the production of delayed type 2 contractions, one or more of the type 1 components seems to be omitted. It is possible that the type 2 contractions, which alone, in our tracings, present the picture of running waves, may be produced by summation of type 1 contractions, and that the manner of this summation, with repression of one or more of the type 1 contractions, determines direction of the larger wave.

We find additional evidence for the larger rôle played by apparently local conditions in the production of pressure waves in the colon, in the occurrence of waves which are directed orally for a portion of their course, anally for a second portion, and which fail to show direction for the remainder.

Zondek (5) has described haustral segmentation in the rabbit's colon as peristalsis alternating with anti-peristalsis in irregular order. It is possible that we are dealing here with this sort of motor activity. However, our records show that the entire colon is capable of acting as a unit, and that frequently the entire proximal or distal half pulsates rhythmically; that this is the usual condition in the distal colon; and that in both proximal and distal colon this type of activity is subject to sudden alteration to form pressure waves which move either oralward or analward.

Holzknecht (9), working on the human colon with the fluoroscope, described haustral segmentation as being opposed to the propulsion of fecal material, and inhibited by the appearance of the infrequent "large colon movements" which he considered peristaltic. Schwarz (10) also described "small colon movements" whose direction he could not trace, in the human colon. In contrast to Holzknecht, he found the human colon never quiet, but always showing the latter type of activity, except during the appearance of the larger movements, which he considered not peristaltic, but massive contractions which forced material out of large segments. Balloon records, by the single balloon method, were obtained by Ganter and Stattmüller from the proximal colon in a patient with a colostomy due to ulcerative colitis (11). The balloon moved analward at the time it recorded high waves occurring in groups, which these observers considered identical with the large contractions of Holzknecht. Peiper, however, working on the distal colon of children with a double balloon inserted through the rectum, found no peristalsis in the normal colon, although it was frequently found in cases of diarrhoea (12).

He considered the activity which he recorded as pendular or stationary, and observed that in the cases in which peristalsis occurred in this region it was followed by a long quiet period. The two latter observations on the human colon, the first showing occasional or periodic peristalsis in the proximal portion, the latter a predominance of stationary activity in the distal portion, we consider to be in agreement with our observations on the dog, although each of these workers deduced similar activity to be characteristic of all parts of the human colon.

The observation of Schwarz (10) that in cases of human constipation the proximal colon was more active than normally, was interpreted by him as the cause of the constipation, the greater activity resulting in resorption of greater amounts of water than in the normal. In the light of our work, an additional factor might be derived. We have observed, in general, on the dog, that high tone and activity in either proximal or distal colon is associated with reduced activity in the other segment. It is clear, however, that the character of activity, at least in the proximal colon, is an important factor in determining the degree and duration of depression in the other segment. Contractions which show progressive delay toward the anus, when they begin to appear in the proximal colon, usually occur simultaneously with depression in the distal colon. When contractions delayed in the same direction appear in the distal colon, there is an immediate shift of direction of delay in contractions in the proximal colon, sometimes to rhythmic pulsations, sometimes to contractions delayed progressively toward the cecum. With this shift in direction, there is depression in tone, and usually, diminution in the substituted activity in the proximal colon.

On the other hand, rhythmic pulsations, either type 1 or type 2, frequently occur throughout the entire colon, or in either segment without affecting the activity of the other. Contractions delayed progressively oralward frequently appear in the proximal colon without alteration of the activity of the distal.

It is possible that this is the descending inhibition preceding peristalsis observed by Bayliss and Starling (2). We are unable, however, to trace a descent of the inhibition. Such inhibition frequently is entirely absent in the proximal colon preceding a period of activity. In the distal colon, rather than finding a progressive or descending inhibition, we usually find either that the entire distal colon is inhibited at the same time, or that the most distal segment shows the first and most complete signs of inhibition. We are totally unable to apply such a mechanism to the apparent effect of distal colon activity upon the proximal colon. In this case there is no suggestion of a progressive inhibition. The effect on the upper balloons is immediate and simultaneous rather than progressive.

The writers are indebted to Dr. A. J. Carlson, under whose direction this work was conducted.

SUMMARY

1. The contractions of the dog's colon are classified under three general types.

2. The relationships of the three types of contractions are such as to suggest that larger contractions are the result of some sort of summation of the smaller.

3. The fundamental activity of the dog's colon is described as rhythmic pulsations. The mechanism of conversion of these pulsations into moving waves is considered.

4. Differences in activity are noted in the proximal and distal colon: The proximal colon shows high tone changes, coinciding with periods of activity, with progressive delay of contractions analward during the first half of the period, and rhythmic pulsations during the middle of the period which may be replaced by contractions delayed oralward in a small area near the cecum toward the end of the period. The distal colon shows only slight tone changes, with little tendency toward interruption of simultaneity in the rhythmic pulsations.

5. A reciprocal relationship is observed between certain types of activity in the proximal and the distal colon.

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THE EFFECT OF ESTRIN ADMINISTRATION ON THE REPRODUCTIVE AND BLOOD VASCULAR SYSTEMS: THE THYROID, THYMUS, HYPOPHYSIS, ADRENALS, KIDNEYS, LIVER AND SPLEEN[1]

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In a previous publication reports were made of the effects of daily estrin administration in the normal and castrate adult bitch. The observations at that time were limited to the basal metabolism; changes in the uterus and external genitalia and the mating and maternal instincts. The present report deals primarily with histological changes in various parenchymatous organs of the immature dog after prolonged administration of estrin.

METHOD AND PROCEDURE. Four puppies (3 females and 1 male) 7 to 9 weeks old at the beginning of the experiment received subcutaneous injections of estrin. These belonged to three different litters. Litter 1 (mongrels) consisted of one experimental female and 1 litter mate control; litter 2 (pedigreed bull terriers) consisted of two experimental animals (1 male and 1 female), and 1 control. Both of these litters were 8 weeks old at the beginning of the hormone administration, and each of the 3 experimental animals received daily doses of 400 rat units of estrin (D'Amour and Gustavson) subcutaneously for 6 consecutive weeks (through the 9th to 14th weeks after birth). One ovary and a portion of the left uterine horn of each experimental female of these two litters were removed for histological examination 10 days before beginning the estrin injections (July 10, 1930). At the end of the experimental period the female dogs were killed. The remaining ovary, a portion of the contralateral uterine horn and the hypophysis were sectioned for histological examination. Litter 3 consisted of one mongrel female (F.); a mongrel puppy of the same age but of a different litter, living in an identical environment and receiving the same kind of dietary, served as a control for this experimental animal. Beginning during the 7th week after birth, April 29, 1930, to May 3 this dog received 25 rat units of estrin daily. May 3 to

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June 4, the daily dosage was increased to 50 rat units; June 4 to June 13 the animal received 400 rat units daily, and from June 13 to September 8, 800 rat units were given daily. On July 10, 1930, one ovary and a piece of the uterus was removed for microscopic examination. On September 8 the animal was killed and gross and histological examinations were made of the remaining ovary; the uterus, spleen, liver, thymus, thyroid, adrenals, heart, aorta and hypophysis.

RESULTS AND DISCUSSION. During the entire experimental period (6 to 17 weeks) these four dogs apparently suffered no general ill effects from the large daily amounts of estrin given. Their appetites, responsiveness to their environment and evidence of general well being and happiness were apparently the same as those of the control litter mates. All puppies grew during the experimental period but at the end of the period of injection the controls were noticeably larger than the injected litter mates. Dog F (litter 3) gained 0.9 kilo in weight from June 25 to September 8. During this time the animal was receiving 800 rat units daily. The testes of the experimental male dog did not descend during the experimental period. But this dog was only 14 weeks old when administration of the hormone was discontinued and the testes of many male puppies raised in the laboratory have not descended at that age. Swelling of the external genitalia in the female puppies was even more marked than was previously described for the normal and castrate adult bitches. And, as in the adult females, the swelling became apparent on the 3rd to the 6th day of hormone administration and persisted for several days after the hormone was discontinued. The following differences in response to estrin between the puppies and the adult dogs were noted: In the puppies no hemorrhagic discharge from the vaginae was observed; questionable or no sexual interest was seen in the normal adult male dogs when in the presence of the puppies receiving estrin for only 6 weeks. But sex interest seemed apparent in these adult male dogs when in the presence of dog F, during the latter part of her experimental period.

The first ovary was removed from the 3 experimental females at the same time (July 10, 1930). At this time the females of litter 1 and 2 were normal dogs 6 weeks old weighing 920 grams and 1282 grams respectively (estrin injections had not yet been instituted). Dog F (litter 3) had been receiving hormone as previously described. Her body weight was 4.3 kilos. The ovary removed from this dog was less than one-half the size of the ovaries of the females of litter 1 and 2 despite the greater size and age of this animal. The remaining ovary of each female was removed at autopsy September 8. At this time the second ovary of dog F was so small that no attempt was made to separate it from the suspensory ligament and fimbriated end of the tube until it had passed through the clearing fluid in the process of preparation for histological examination. The gross appearance

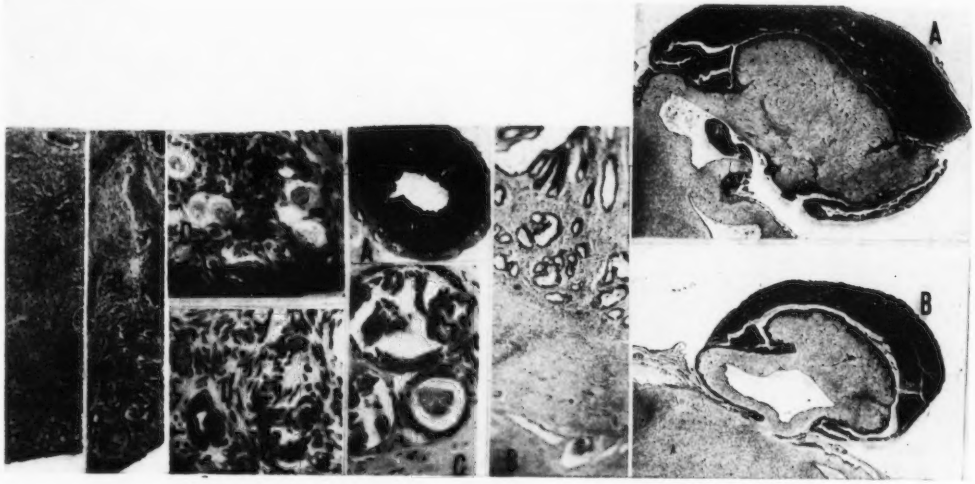

Fig. 1 Fig. 2 Fig. 3

Fig. 1. (Reduced to one-third natural size.) Portion of cross sections through the ovaries of a control dog, 5 weeks old, and dog F, 7 months old, after 17 weeks of daily estrin injections. Both ovaries were serially sectioned. The illustrations are as nearly through the same levels as could be estimated. *A* and *C* = control dog; *B* and *D* are estrin injected dog. *A* and *B* = × 90; *C* and *D*, × 500. Note the increased amount of rete ovarian and the decreased number of primordial follicles in B. The relative increase in stroma and the wide space between the primordial follicles are indicated in *D*.

Fig. 2. (Reduced to one-third natural size.) Cross sections through the uteri of the same dogs mentioned in figure 1. *A* = uterus of normal dog 7 weeks old (× 90). *B* = dog F after daily doses of estrin for 17 weeks (× 90). *C* = high power magnification of portion of B showing details of hypertrophy of the basal glands (× 500).

Fig. 3. (Reduced to one-third natural size.) Comparison of sagittal sections near the same level of *A*, control dog, and of *B*, dog F after 17 weeks of hormone administration. These dogs were the same age. Weight of control = 5.5 kilos. Weight of dog F = 5 kilos. Magnification of both sections = × 30. Note the smaller size of section *B*, also the failure of closure of the infundibular portion of the 3rd ventricle.

of the ovaries removed from the females of litter 1 and 2 were much smaller than the ones removed before beginning the estrin injections. This decrease in the size of the ovaries of the dog, following estrin administration, conforms to the effect of estrin on the ovaries of rats reported by Meyer, Leonard, Hisaw and Martin (1930).

Histologically, at the end of the experiment all ovaries contained relatively much more ovarian stroma than before injection, and no maturing follicles were seen, even on serial sectioning, of the ovary of the dog approximately 7 months old (see fig. 1, *D*). The primordial follicles in the experimental animals seemed fewer in number and much farther apart than before beginning the experiment (see fig. 1, *A* and *B*). There was much more rete ovarian in the ovaries removed after estrin injections than before administering the hormone. Atretic follicles were seen both under normal and experimental conditions.

The uteri of all 3 dogs became very large as a result of the estrin injections. A comparison of figure 2, *A* and *B*, shows the histological changes resulting from the hormone administration. The enlargement of the uterine glands, with special reference to the basal glands, is most pronounced but no mitotic figures were seen.

The hypophyses of the 3 experimental females, and of the controls of dog F and of litter 2 were studied histologically by serial sagittal sections. The hypophyses of the dogs receiving female sex hormone for only 6 weeks showed no outstanding changes from that of the control. On inspection of the sagittal sections of dog F variations from her control seemed apparent. In order to objectively determine this, the following measurements were made through definite points and compared to measurements through the same points on a comparably sagittal section of the control. Greatest length through the entire sagittal section of the control in millimeter = 4.9. In dog F = 3.7; greatest length of the anterior lobe of the control = 4.9; of dog F = 3.5. Greatest width of this lobe over the infundibulum in the control = 1.4, in dog F = 1.0. Variations of the remainder of this lobe of the control = 0.5 to 1.0, of dog F = 0.3 to 0.6. These figures point to a markedly smaller hypophysis in the experimental animal than in the control. But we do not wish to infer that any significance can be placed upon these values until the variations in size of a large number of hypophyses of dogs of the same age and size have been established. Whether this variation is within the limit of a normal range or not, the fact remains that there is much more hypophysis present in dog F than has been demonstrated in many experimental animals showing no hypofunction of the hypophysis after partial removal of the gland; or in clinical cases where much of the gland is replaced by pathological formations and still no clinical evidence of hypopituitarism exists. The chromophobes of the anterior lobe in some places give histological evidence of cellular exhaustion. But we question the advisability of placing any functional significance to this.

A developmental anomaly of rare occurrence in the dog, i.e., failure of the infundibular portion of the 3rd ventricle to close, was observed in dog F (fig. 3, *B*). This evidently has no bearing in the experimental phase of this work inasmuch as it was not observed in the other dogs. Mention of this is justifiable here only because of its rare occurrence.

The thyroid of dog F, at autopsy, was small, both lobes weighing 1.2 gms. (the animal weighed 5 kilos). Histologically, the alveoli were lined by tall cuboidal to columnar epithelial cells with large round nuclei usually located in the lower half of the cell. The lumen of the alveoli were generally large and penetrated by folds of epithelium from the alveolar walls, many alveoli having several folds as occurs in typical papillary hyperplasia. The colloid content in general is extremely scarce, the majority of the large lumen being nearly empty. The few lumen, containing colloid, illustrate the substance as faintly acidophilic with large vacuoles near the periphery. There are a few alveoli with lumen completely distended by colloid. Mitotic figures are absent. This hyperplasia of the thyroid conforms quite closely to that which Marine (1915) described as occurring in about 30 per cent of normal young dogs in the Great Lakes region of this country. The capillaries outside the alveoli are moderately congested. No evidence of arterial sclerosis was demonstrable in the thyroid arteries.

The thymus, grossly, seemed large. Histologically it appeared lobulated and of the infantile type. Grossly the kidneys appeared normal. Histologically the glomeruli seemed normal. The tubules contained a small amount of protein material, the significance of which cannot be interpreted at this time since occasionally a normal dog, killed with ether, presents similar histological findings. The condition, however, suggests the possibility of protein in the urine as a result of estrin administration. This phase of the experiment needs further investigation. The liver was essentially normal with occasionally slight congestion about the center veins. Neither gross nor histopathology could be detected in the adrenals, spleen, heart or aorta. The nipples of both male and female dogs became large.

Beginning with the 8th week of estrin injections, a peculiar mal-occlusion of the jaw became noticeable in dog F. This seemed to be the result of a more rapid growth of the maxilla than of the mandible, so that typical maxillary prognathism with spreading and over-lapping of the teeth was in evidence. Since only one animal (dog F receiving estrin over 17 weeks) displayed this condition, it is impossible to state whether it resulted from the hormone or whether it is an accidental developmental phenomenon. It is quite possible that it is due to the latter since none of the other dogs developed this condition, but the possibility of its being related to the conditions already described in the anterior lobe of the hypophysis should not be overlooked at this time.

SUMMARY

1. Subcutaneous injections of estrin over a period of time varying from 6 to 17 weeks caused a diminution of the size of the ovaries, an arrest in the development of the follicles and a relative increase in the amount of ovarian stroma.

2. Marked hyperplasia of the uterine glands (especially the basal glands) occurs in puppies after estrin injections but no hemorrhagic discharge from the vagina was observed.

3. The hypophysis of the dog receiving female sex hormone for 17 weeks was smaller than the hypophysis of the control and measurements indicate a relatively greater decrease in the anterior than in the posterior lobe.

4. Neither gross nor histopathology was observed in the arteries, heart, liver, spleen, thymus or adrenals after 17 weeks of daily doses of female sex hormone.

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THE PHYSIOLOGY OF THE LIVER

XIX. The Utilization of Fructose Following Complete Removal of the Liver

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The symptoms of hypoglycemia which invariably follow complete removal of the liver are closely correlated with the decreased amount of glucose in the blood, and glucose is specific for relief of these symptoms. Glucose, or other sugars such as maltose, mannose, and glycogen, which are promptly converted into glucose by the blood, restore the hypoglycemic animal to a normal condition within a few minutes after intravenous administration. Mann and Magath (1922) found that fructose and many other sugars administered intravenously failed to give immediate relief in this condition of hypoglycemia. Later, in the course of studies of the respiratory exchange of hepatectomized animals, it was found that fructose increases the respiratory quotient and the metabolic rate. This appeared to be definite evidence that the liverless animal is capable of utilizing fructose, and might readily be accepted as proof of the direct utilization of this substance. The results of our experiments on the utilization of fructose, following complete removal of the liver, indicate that fructose is utilized by conversion to glucose. Symptoms of hypoglycemia following removal of the liver are prevented by administration of fructose if it is given sufficiently early to allow time for its conversion into glucose.

The liver has long been regarded as being intimately concerned with the metabolism of fructose, since in many patients with hepatic disease lessened tolerance for this sugar has been discovered. Mann and Bollman (1926) found that in hepatic insufficiency experimentally produced, the tolerance for intravenously injected fructose was not altered, and that even in complete absence of the liver the total sugar content of the blood decreased at a rate similar to that in a normal animal after fructose was administered. These observations do not indicate that the liver is not involved in the normal metabolism of fructose. Cori (1926) found that administration of fructose by the intestinal route produced deposition of glycogen in the liver of rats as rapidly as when glucose was given. In his experiments maximal retention of glycogen occurred in four hours, and

amounted to 39 per cent of the fructose as compared to 17 per cent of the glucose. Large doses of insulin almost completely suppressed the formation of hepatic glycogen from both fructose and glucose. Cori and Cori (1927) found that fructosuria occurred in rats when fructose was infused into the femoral vein at about half the rate which exceeded the limit of tolerance when the substance was injected into a mesenteric vein. These investigators also confirmed the work of Wierzuchowski (1926); namely, that insulin is without effect on the tolerance for fructose injected intravenously. Later (1927, 1928) they concluded that a surplus of insulin led to an increased rate of oxidation of fructose, and that the rate of deposition of glycogen in the tissues was correspondingly decreased. With glucose, however, insulin was found to increase the rate of deposition of glycogen in the peripheral tissues.

Methods. The fructose content of the blood was determined by the method of Campbell and Hanna. This method permits good recovery of fructose added to blood in small amount, comparable to the concentrations of fructose with which this paper is mainly concerned. With concentrations of more than 300 mgm. for each 100 cc., however, it was found advisable to repeat the determinations and blanks, using smaller amounts of the blood filtrate. This was done because of the rather indefinite end point for the titration with permanganate when the volume used in titration became large. This method is also satisfactory for the determination of fructose in the urine, when large amounts are present, so that a very dilute urine may be used. The total sugar content of the urine was determined by the method of Benedict.

The total sugar content of the blood was determined by the method of Folin, and the glucose content of the blood was taken by difference after subtracting the amount of fructose present.

The glycogen content of muscle was determined by the method of Pflüger, which was slightly modified to permit the use of specimens of about 1 gram. After isolation of the glycogen it was hydrolyzed with 2 per cent hydrochloric acid at 100°C. for one hour and the glyoegen was determined as glucose. The muscle used for determination of glycogen was obtained at the time of the major operation during ether anesthesia. Subsequent specimens were obtained under local anesthesia produced by procaine. About 2 grams of muscle were obtained for each determination, and the fat and superficial fascia were rapidly removed. The specimen was then cut in two; each portion was rapidly weighed and was placed immediately in 1 cc. of 50 per cent potassium hydroxide at 100°C.

All operative procedures were performed under ether anesthesia with aseptic technic. The method of removal of the liver was that of Mann (1921).

Results. Intravenous injection of fructose, 0.5 gram for each kilogram

of body weight, in normal animals is followed by fairly rapid disappearance of this substance from the blood. Five minutes after injection the concentration of fructose in the blood is found to be between 100 and 150 mgm. for each 100 cc. and after the first thirty minutes only 15 or 20 mgm. remain. An hour after injection the blood usually is found to be free of fructose. Some fructose is found to have been excreted in the urine, but the disappearance from the blood is the same as that which occurs in animals in which bilateral nephrectomy has been performed. The glucose content of the blood is not appreciably altered after injection of this amount of fructose. The injection of larger amounts of fructose may be followed by a slight increase in the glucose content of the blood, but we have not obtained increases sufficient to be considered abnormal or to give rise to glycosuria.

Intravenous administration of fructose in dogs following complete removal of the liver has been without effect if such administration has been delayed until such time as the animal was in the condition of extreme hypoglycemia. The administration of glucose at this time, however, brings about rapid and complete recovery. The injection of fructose in the liverless animals at any earlier time before the extreme stage of hypoglycemia is reached, relieves the symptoms of hypoglycemia and the animals appear normal. The intravenous injection of 0.5 gram fructose for each kilogram of body weight is followed by a disappearance of this substance from the blood. The disappearance is somewhat delayed as compared to that in normal animals, traces of fructose remaining in the blood for two to three hours after injection. The type of curve is similar in the normal and in the liverless animal, and similar amounts of fructose appear in the urine. The glucose content of the blood of liverless animals is definitely increased following administration of fructose. From five to fifteen minutes after injection of levulose the glucose content of the blood has increased from the hypoglycemic level to a level that is approximately normal. Hypoglycemia can be prevented by repetition of the administration of fructose, and liverless animals can be maintained in an apparently normal condition for more than twenty-four hours after removal of the liver. It was also found that the glycogen content of the muscle could be increased in the hepatectomized animal by continued administration of fructose only. In all of these experiments it was found necessary to inject somewhat more fructose than would have been required if glucose had been given, but unless this was done the animals showed the first symptoms of hypoglycemia. The glucose content of the blood was always found to be low when symptoms of hypoglycemia were present, and it was obvious that the fructose content of the blood was not a factor in the prevention of symptoms of hypoglycemia except as the fructose was converted to glucose. The lactic acid content of the blood and of urine of

hepatectomized animals appears to be slightly increased following administration of fructose. We have not been able to satisfy ourselves that this increase is the direct result of administration of fructose, since many other factors, such as anoxemia, acid-base disturbances, and so forth, may bring about equally great changes in the lactic acid content of the blood and urine (figs. 1 and 2).

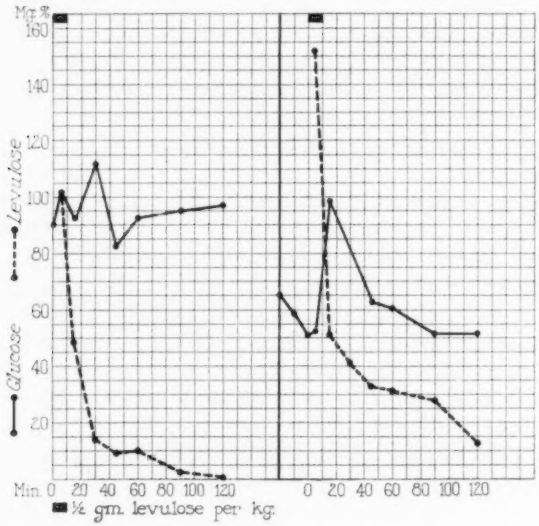

Fig. 1. Administration of fructose increases the glucose content of the blood in the hepatectomized animal. The glucose and fructose content of the blood following intravenous injection of the same amounts of fructose; (left) the injection of 0.5 gram of fructose for each kilogram of body weight of dog 8, November 3, 1926, thirty-five minutes after ether anesthesia; (right) a similar injection in the same animal November 4, 1926, thirty-five minutes after ether anesthesia and removal of the liver. There was definite increase of the glucose content of the blood. Curves similar to that at the right are obtained with injection of fructose into animals deprived of the liver and pancreas, liver and spleen, liver and kidneys, and liver, spleen, pancreas, and kidneys.

In a number of experiments fructose was administered to animals from which all the abdominal viscera had been removed with the liver. These animals usually recover from the anesthetic, and by the frequent administration of glucose easily are maintained in a condition that appears normal for fifteen to twenty hours. Unless glucose is given, hypoglycemia develops and they die within a few hours. Fructose administered to these animals is entirely without effect on the glucose content of the blood or on the symptoms of hypoglycemia. A high concentration of fructose has

been maintained in these animals for several hours, during which time marked symptoms of hypoglycemia have developed several times and have been relieved each time by the injection of glucose. Since these animals do not live long without the administration of glucose, evidence of the conversion of fructose to glycogen in the muscles could not be obtained (fig. 3).

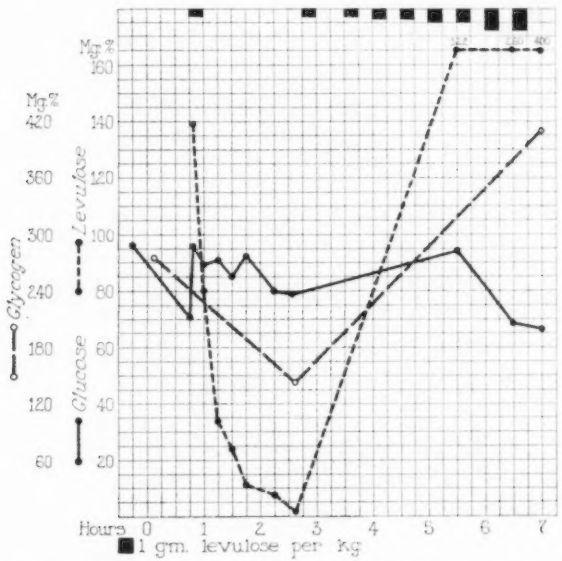

Fig. 2. Administration of fructose prevents hypoglycemia and may increase the glycogen content of the muscles in the dehepatized animal. The changes in glucose and fructose content of the blood and glycogen content of the muscles following repeated injections of fructose in dog 9 after complete removal of the liver. Similar curves are obtained in animals following removal of the liver and pancreas, liver and spleen, and liver, pancreas, spleen, and kidneys.

The results of the administration of fructose following removal of the liver and pancreas were similar to those obtained following hepatectomy alone. That is, increased glucose content of the blood could be demonstrated after administration of fructose, and the animals survived without administration of glucose.

The results of administration of fructose to animals from which the liver and the abdominal portions of the gastro-intestinal tract had been removed were similar to those obtained in animals with complete removal of all the abdominal viscera. That is, there was no appreciable chemical or physiologic effect from the administration of fructose.

The intravenous injection of fructose 0.5 gram for each kilogram of body weight into animals from which the stomach and intestines only had been removed was followed by disappearance of the levulose from the blood in from two to three hours. Less than 10 per cent of the levulose was found to be excreted in the urine, and in animals from which both kidneys were removed at the time of removal of the stomach and intestines, the fructose

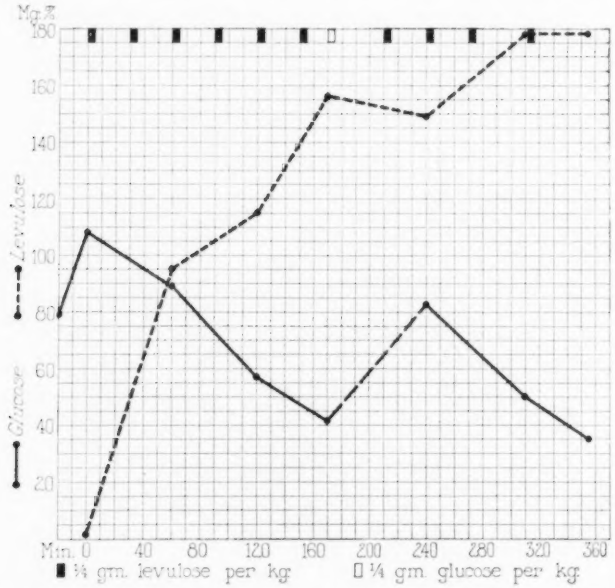

Fig. 3. Administration of fructose does not prevent hypoglycemia following removal of the liver, stomach, and intestines. The changes in glucose and fructose content of the blood following repeated injections of fructose into dog 10 after removal of all the abdominal viscera. Marked symptoms of hypoglycemia developed and were immediately dispelled three hours after operation by the injection of glucose. Later the animal died with definite symptoms of hypoglycemia. The glucose content of the blood of this animal was not altered in any way by the injection of fructose. Curves similar to this are obtained following injections of fructose into animals from which the liver, stomach, and intestines are removed.

disappeared at approximately the same rate. No definite increase in the glucose content of the blood could be noted in these animals, because this content usually was found to be greater than normal without the administration of any sugar.

COMMENT. There can be no doubt that tissues other than those of the liver are able to convert fructose to glucose. The amount of fructose excreted by the liverless animal is similar to that excreted by a normal

TABLE 1

Prevention of symptoms of hypoglycemia in the dehepatized dog by administration of fructose

Reverse Eck fistula November 28, 1924; portal vein ligated May 14, 1925; experiment October 23, 1925, after a fast of eighteen hours; weight of animal 15.6 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD	COMMENT
	Total sugar	
	mgm. for each 100 cc.	
8:45 a.m.	75	Anesthesia began 8:50; liver removed 9:05; specimen of left vastus medialis muscle removed 9:15, which contained 0.147 per cent glycogen; ether discontinued 9:20
3:20 p.m.	180	Intravenous injection of 7.8 grams fructose in 15 cc. sodium chloride solution had been made 9:20; injection had been repeated 10:20, 11:20, 12:20, 1:20, 2:20
4:20	132	Injection had been repeated 3:21
11:00	241	Injection had been repeated 4:21, 5:30, 6:30, 7:30, 8:30, 9:30, 10:30; animal died 11:00, specimen of right vastus lateralis muscle contained 0.220 per cent glycogen; no symptoms of hypoglycemia were observed during the entire course of the experiment

TABLE 2

Prevention of appearance of symptoms of hypoglycemia in the dog by administration of glucose following removal of the abdominal viscera; administration of fructose without effect

Partial ligation of inferior vena cava January 29, 1925; experiment January 7, 1926, after a fast of eighteen hours; weight of animal 10 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD	COMMENT
	Total sugar	
	mgm. for each 100 cc.	
9:30 a.m.	81	Anesthesia began 9:30; all abdominal viscera and kidneys removed 10:00; ether discontinued 10:10
11:00	123	Intravenous injection of 5 grams fructose in 25 cc. sodium chloride solution had been made 10:00
11:40	215	Injection had been repeated 11:01; marked symptoms of hypoglycemia had been noted 11:30; weakness, muscular twitchings and coma 11:40; animal appeared to be dying at 11:40; intravenous injection of 5 grams glucose in 10 cc. sodium chloride solution 11:40; animal appeared normal 11:45, intravenous injection of 5.0 grams fructose in sodium chloride solution 12:00, 1:00, 2:00 and 3:00; animal died with typical symptoms of hypoglycemia 3:05

animal following the administration of similar quantities of fructose. The rate of disappearance of fructose from the blood is only slightly less in the

TABLE 3

Utilization in the dog of large amounts of fructose to replace glucose after hepatectomy; apparent conversion of fructose to glucose

Reverse Eck fistula, June 4, 1929; portal vein ligated November 26, 1929; experiment August 7, 1930 following a fast of eighteen hours; weight of animal 13.5 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD				URINE*		COMMENT
	Glucose	Fructose	Carbon dioxide	Lactic acid	Fructose	Lactic acid	
	mgm. for each 100 cc.	*mgm. for each 100 cc.*	*per cent by volume*	*mgm. for each 100 cc.*	*mgm. for three hour period*	*mgm. for three hour period*	
8:30 a.m.	88		40.4	7.2			Anesthesia began 8:30 a.m.;
9:30	98		26.2	10.4			liver removed 8:50; ether
12:30 p.m.	60	11	37.6	23.8	1,113	70.2	discontinued 9:00; urinary bladder washed 9:30; intravenous injection of 13.5 grams fructose in 50 cc. sodium chloride solution 9:30; injection repeated 11:00
3:30	75	10	37.6	17.1	864	162.5	Injection had been repeated 12:31, 2:00
6:30	69	Trace	38.1	10.3	963	185.1	Injection had been repeated 3:31, 5:00
9:30	51	Trace	35.3	30.5	1,071	220.2	Injection had been repeated 6:31, 8:00
12:30 a.m.	73	Trace	32.4	29.3	674	115.2	Injection had been repeated 9:31, 11:00
3:30	72	2	31.5	27.0	610	160.2	Injection had been repeated 12:31, 2:00
6:30	74	18	29.6	42.3	612	118.0	Injection had been repeated 3:31, 5:00
9:30	88	20	20.4	35.9	578	94.6	Injection had been repeated 6:31, 8:00
12:00 m.	83	24	14.7	39.2			Injection had been repeated 9:31, 11:00;† animal anuric; died 12:00 m.

* The urine contained 6.485 grams fructose and 1.126 grams lactic acid.

† In all, 243.0 grams of fructose were injected.

liverless animal. Finally, there is the definite observation of increased glucose in the blood of hepatectomized animals following the administration of fructose and also the increased glycogen content of the muscles.

We were somewhat surprised to find that, in the absence of the liver, the stomach and intestines were necessary for the conversion of fructose to glucose. This conversion has been demonstrated following removal of the liver, spleen, pancreas, and kidneys, but always was definitely absent when the stomach and intestines were removed with the liver. We have no definite data regarding the nature and mechanism of the conversion. The interval of time between the injection of fructose and the appearance

TABLE 4

*Conversion of fructose to glucose in the dehepatized depancreatized dog**

Reverse Eck fistula June 25, 1929; portal vein ligated, December 2, 1929; experiment February 10, 1930 after a fast of eighteen hours; weight of animal 13 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD		COMMENT
	Glucose	Fructose	
	mgm. for each 100 cc.	*mgm. for each 100 cc.*	
8:30 a.m.	76		Anesthesia began 8:35 a.m.; liver removed
9:35	58		8:55; pancreas removed 9:10; ether discontinued 9:15; animal weighed 12.3 kgm. postoperatively
9:40	54	196	Intravenous injection of 12.3 grams fructose in 50 cc. sodium chloride solution had been made 9:36
10:05	60	128	
10:20	78	96	
10:35	84	68	
11:05	96	214	Injection had been repeated 10:36
11:35	92	130	
12:05 p.m.	63	300	Injection had been repeated 11:36
12:35	66	242	
1:35	122	222	Injection had been repeated 12:36
2:25	124	232	Injection had been repeated 1:36
3:35	74	400	Injection had been repeated 2:36
4:35	88	488	Injection had been repeated 3:36
4:50	65	695	Injection had been repeated 4:36

* Symptoms suggestive of hypoglycemia were not observed at any time.

of an increased concentration of glucose in the blood is not long, but it is sufficient to allow for conversion of the sugar in the vascular endothelium of the intestine, or in the cells of the intestine, or even for the excretion of fructose into the intestinal lumen and its resorption and conversion into glucose. Since the liver also appears to be able to convert fructose to glucose, we are inclined to the belief that the mucosal cells of the intestine are responsible for the conversion in the intestine.

In the absence of the liver the dog is not able to utilize fructose directly

so that such utilization, if any, reflects any changes in the rate of utilization of glucose. If symptoms of hypoglycemia did not develop in the liverless animal that was subjected to injection of fructose, and if life was prolonged beyond what would be expected without administration of glucose, it was always found that the glucose content of the blood had been maintained above a hypoglycemic level. In the animals from which the stomach,

TABLE 5

*Fructose not utilized in the absence of the liver and intestines in the dog**

Reverse Eck fistula, June 25, 1929; portal vein ligated December 2, 1929; experiment February 13, 1930, after a fast of eighteen hours; weight of animal, 12.8 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD		COMMENT
	Glucose	Fructose	
	mgm. for each 100 cc.	*mgm. for each 100 cc.*	
8:30 a.m.	85		Anesthesia began 8:35 a.m.; liver removed 8:55; stomach and intestines removed at 9:10; other abdominal viscera appeared in good condition; ether discontinued at 9:15; dog weighed 11.5 kgm. post operatively
9:20	74		
9:50	59	123	Intravenous injection of 11.5 grams fructose in 50 cc. sodium chloride solution had been made 9:21
10:20	43	93	
10:50	40	174	Injection had been repeated 10:21
11:20	39	127	Slight muscular twitching observed
12:30 p.m.	35	145	Injection had been repeated 11:21; numerous muscular twitchings and marked weakness
1:30	23	163	Injection had been repeated 12:31; extreme symptoms and coma of hypoglycemia; intravenous injection of 11.5 grams glucose at 1:30; animal completely recovered in five minutes
2:40	29	90	Animal died at 2:40 with typical symptoms of hypoglycemia

* A total amount of 46 grams of fructose was injected. During the course of the experiment the animal passed 800 cc. of urine which contained 35.0 grams of fructose.

intestines, and liver were removed, symptoms of hypoglycemia occurred, irrespective of the administration of fructose or of the fructose content of the blood, but the symptoms were definitely referable to the diminished glucose content of the blood and were promptly dispelled following administration of glucose. The increases in glycogen content of the muscles

TABLE 6

Apparent utilization of fructose in the presence of the liver but in the absence of the stomach and intestines in the dog

April 14, 1930, after a fast of eighteen hours weight of animal was 6.8 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD		COMMENT
	Glucose	Fructose	
	mgm. for each 100 cc.	*mgm. for each 100 cc.*	
8:30 a.m.	133		Anesthesia began at 8:30 a.m.; stomach and intestines from juncture of esophagus and stomach to the lower end of the sigmoid removed at 9:00; kidneys removed at 9:05; other abdominal viscera appeared in good condition; ether discontinued at 9:15; animal weighed 6.4 kgm.
10:00	237		
10:05	261	215	Intravenous injection of 3.2 grams fructose in 20 cc. of sodium chloride solution had been made 10:01
10:15	306	106	
10:30	125	79	
10:45	120	41	
11:00	137	28	
11:30	85	12	
12 m.	90	5	
2:00 p.m.	111		

TABLE 7

Utilization of fructose in the nephrectomized dog

October 12, 1926, after a fast of eighteen hours weight of animal was 6.0 kgm.

TIME OF TAKING BLOOD	JUGULAR BLOOD		COMMENT
	Glucose	Fructose	
	mgm. for each 100 cc.	*mgm. for each 100 cc.*	
8:50 a.m.	74		Anesthesia began 9:30; kidneys removed at 9:35; ether discontinued 9:40
9:50	105		
9:55	103	135	Intravenous injection of 3.0 grams fructose in 20 cc. sodium chloride solution had been made 9:51
10:05	132	48	
10:20	120	17	
10:35	93	14	
10:50	82	12	

following the administration of fructose are also probably due to the increased glucose content of the blood, not to the direct conversion of fructose to glycogen. When the conversion of fructose to glucose was prevented by removal of the gastro-intestinal tract and liver, the glycogen content of the muscles was found to decrease as rapidly as when fructose was not given.

In view of the recent observations of Rose, Giragossintz, and Kirstein: namely, that injection of fructose into the small intestine gave rise to an increased lactic acid content of the portal blood, we investigated the lactic acid content of the blood following administration of fructose to liverless animals. The lactic acid content of the blood and urine was found to be increased after administration of fructose. This increase is slight compared to the amounts of fructose administered or to the amount of glucose formed from the fructose. Since the concentration of lactic acid is increased in the blood and urine following reduction of the carbon dioxide combining power of the blood, and also after short periods of anoxemia, the slight increases in lactic acid which we have observed do not seem of significance. It does not appear that the formation of lactic acid is an intermediate step in the conversion of fructose to glucose in the liverless animal because these animals do not show any ability to utilize lactic acid to make glucose or to prevent hypoglycemia.

PROTOCOLS. Only one protocol is given for each type of experiment performed in this series. Several experiments of each type were performed, but in no case were the results markedly different from the experiments which are abstracted here (tables 1 to 7).

SUMMARY

The injection of fructose into animals following complete hepatectomy is followed by a rise in the glucose content of the blood, and symptoms of hypoglycemia are prevented. If the stomach and intestines also are removed with the liver, conversion of fructose to glucose does not take place and the animal succumbs to hypoglycemia unless glucose is administered. Removal of the other abdominal viscera does not affect this conversion. In animals with intact livers but from which the stomach and intestines are removed, the process of converting fructose to glucose apparently is possible.

Fructose is not utilized directly in the absence of the liver but is converted to glucose. The conversion of fructose to glucose may be accomplished in the intestines or in the liver but not to any appreciable extent in the other tissues of the body.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XX. The Detoxicating Function of the Liver with Special Reference to Strychnine

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In recent years the detoxicating function of the liver has been attracting considerable interest among clinicians as well as physiologists. Much of the work which has been done on this subject, however, has yielded questionable data because of the unphysiologic nature of the experiments, the unreliable quantitative methods employed, and the inadequacy of the control observations. From many points of view it appeared to us desirable to evolve suitable methods of studying the detoxicating function of the liver.

In order to facilitate this work we investigated the ability of the liver to destroy strychnine, a stable, readily detectable substance. The biologic assay used for the quantitative estimation of strychnine in blood, muscle and hepatic tissue has been described (9). Suffice it to say here that this method was thoroughly tested and found to give consistent results with a maximal error of 15 per cent. This error was invariably on the low side; that is, the estimated concentration of strychnine was slightly lower than the actual concentration. Correction was not made for this error, as a routine. Any other poison that produces marked physiologic effects in the organism and that is readily estimated in biologic material could have been employed. From a study of the ability of the liver to detoxify this substance we have come to a number of conclusions regarding the experimental methods that previous workers have used for investigating the problem, and we have been able to postulate certain requirements which must be fulfilled before the detoxication of any given compound can be attributed to the liver.

In the literature, one finds approximately twenty different procedures that have been employed in studying hepatic detoxication. Of the six methods employed in this research, the first three are considered mainly because of the frequency with which they have been utilized by previous workers, and not because it is thought that they yield data suitable for

definite conclusions. The other methods employed, although not new in principle, are described more in detail, since they are believed to be particularly adaptable for studying the ability of the liver to detoxify various poisonous substances. Dogs were used exclusively, and all operative procedures were performed under ether anesthesia. The methods of study were as follows:

1. Comparison of dogs with Eck fistula and normal dogs.
2. Comparison of the effectiveness of strychnine injected into the peripheral vascular system, and injection into the portal vein.
3. The ability of incubated pulp of liver to destroy strychnine.
4. Estimation of strychnine introduced into the circulation of a heart-lung-liver perfusion, compared with that introduced into a heart-lung-hind-limb preparation.
5. Susceptibility of normal and dehepatized dogs to strychnine.
6. Rate of disappearance of strychnine from the blood stream of normal and dehepatized dogs.

COMPARISON OF DOGS WITH ECK FISTULA AND NORMAL DOGS. The susceptibility of dogs to strychnine, before and after anastomosis of the portal vein with the vena cava, was found to differ only slightly, whereas other definite evidence indicated that the liver formed a large share of the body's defense against this alkaloid. Consequently we do not believe that the dog with an Eck fistula is a proper subject for the accurate study of hepatic detoxication.

COMPARISON OF THE EFFECTIVENESS OF STRYCHNINE INJECTED INTO THE PERIPHERAL VASCULAR SYSTEM, AND INJECTION INTO THE PORTAL VEIN. Comparing the effectiveness of a toxic product when injected into a peripheral vein, in contrast to the portal vein, was one of the first procedures used in a study of the detoxifying function of the liver (1). The technic of the method is simple. The degree of reaction caused by the injection of a known amount of a poison into the femoral artery is first determined. Several days later, in order to avoid any cumulative effect of the toxin, the abdomen is opened aseptically under local anesthesia and the same amount of the toxin is introduced at the same rate into the portal vein. Dilute solutions must be injected slowly, if any difference is to be noted between the two reactions.

We have found that the "just convulsive" dose of strychnine for a dog is slightly smaller after injection into the femoral artery than after introduction directly into the portal vein. This difference was never found to be marked.

It is necessary to point out one valid objection to the technic employed by most workers who have used this method; namely, injection into a peripheral vein does not constitute a fair control for the injection into the portal vein, since in the former instance a capillary bed is not traversed by

the material injected before it reaches the general circulation. In the present study, control injections were made into the femoral artery rather than into the femoral vein.

THE ABILITY OF INCUBATED PULP OF LIVER TO DESTROY STRYCHNINE. Many conflicting data have been published concerning the action of incubated pulp of liver on various poisons. Most authors who have noted the destruction of a poison under such conditions have inadequately controlled the quantitative extraction of the toxin from the pulp. From our experience we do not regard this as a satisfactory method of study.

ESTIMATION OF STRYCHNINE INTRODUCED INTO THE CIRCULATION OF A HEART-LUNG-LIVER PERFUSION, COMPARED WITH THAT INTRODUCED INTO A HEART-LUNG-HIND-LIMB PREPARATION. Perfusion experiments were first used in studying the detoxicating function of the liver more than fifty years ago (2). The objections usually raised against experiments on perfusion of the liver are probably well founded in most cases. It might be worth while to mention, however, that these criticisms regard the unphysiologic aspects of the experiments, such as an inadequate flow of blood, injured hepatic cells, altered metabolism, deficient oxygenation, and unphysiologic perfusion fluids. Consequently, if well controlled evidence of the ability of the liver to render certain poisonous substances innocuous, during the course of their repeated passage through this organ, can be obtained despite the existence of these unphysiologic conditions, it seems as though some significance must be attached to the results. It is our belief that many of these conventional objections have been largely overcome, however, by the use of Starling's heart-lung preparation as a pump for perfusion of the liver.

Method of procedure. In general, three different series of profusion experiments were performed. In each instance a known amount of strychnine was added to the perfusion fluid and quantitative studies were then made to determine the rate of disappearance of the drug from the circuit. Studies were first made on the heart-lung circuit by itself. In a second series of experiments, similar observations were made in the heart-lung-liver preparation. Finally, heart-lung-hind-limb perfusions were used as controls. All operative procedures were performed under ether anesthesia.

In more detail, the procedure which we employed in performing these perfusion studies is as follows: a heart-lung circuit is established, preferably on a small dog. Defibrinated blood serves as the perfusion fluid. Prior to connecting the cannula in the superior vena cava with the tube coming from the venous reservoir, a small amount of heparin is allowed to enter the right side of the heart. This anticoagulant temporarily renders the dog's blood incoagulable. Immediately after ligation of the aorta, the abdomen is opened and the liver is exposed. Cannulas are inserted

into the portal vein, the hepatic artery, and the common bile duct in rapid succession. The liver is removed and placed at once in a warm container so situated that it drains directly into the venous reservoir (fig. 1). The hepatic artery and portal vein are connected proximally and distally to the artificial peripheral resistance, respectively. Connecting these vessels in this manner gives a portal pressure of approximately 15 mm. of mercury and a pressure in the hepatic artery ranging from 125 to 140 mm. of mercury. If desired, the entire flow of blood can be shunted through the liver by tightening the burette clamp on the rubber tube leading to the

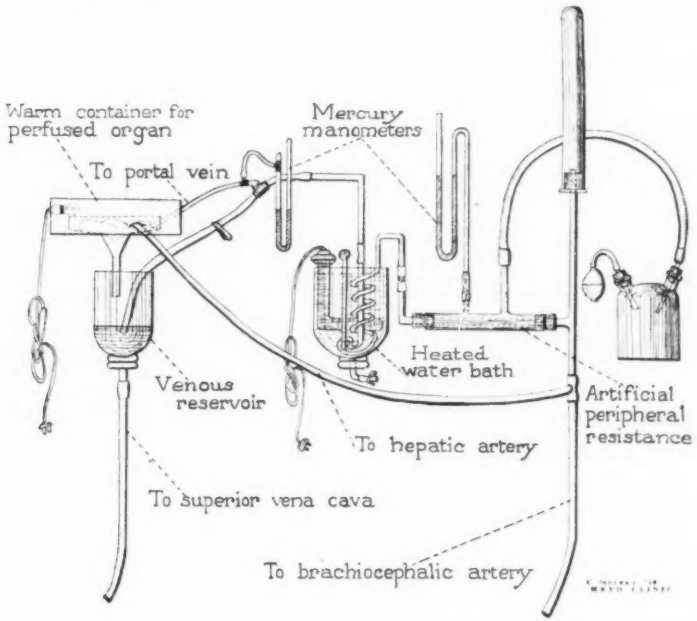

Fig. 1. Artificial circulation used in perfusion experiments

venous reservoir. This will afford a minute flow of blood from the liver of 250 to 400 cc. The cannula in the common bile duct is introduced into a small collecting tube. The drug to be studied is now added to the venous reservoir and specimens of liver and blood for quantitative analysis are removed at varying intervals.

The procedure followed in establishing a heart-lung-hind-limb circuit is the same as that used for perfusion of the liver, with a few minor differences. After one of the hind legs is shaved and cleansed, a heart-lung circuit is established according to the usual technic. A cannula is then

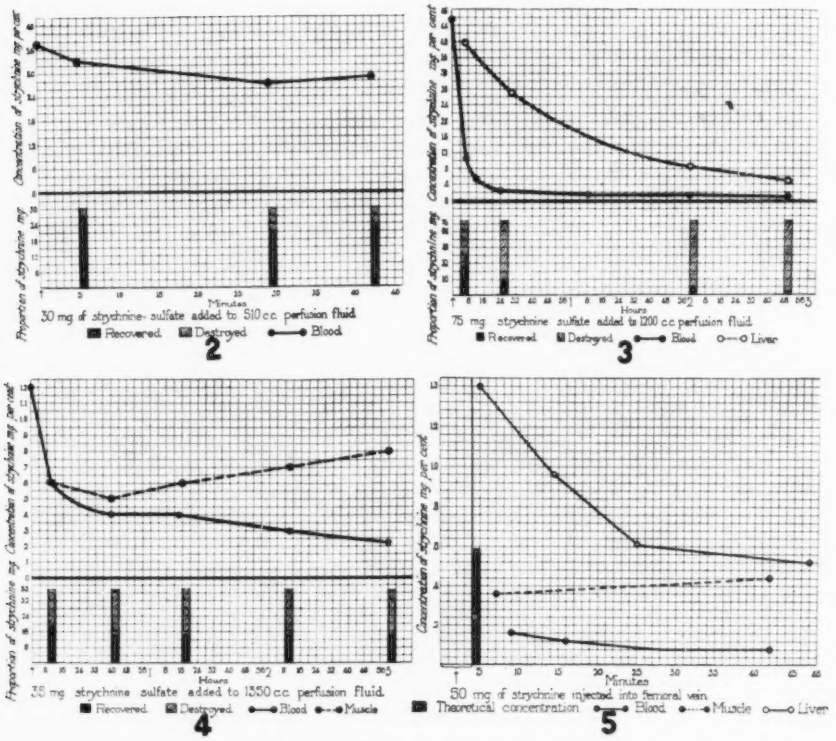
30 mg of strychnine sulfate added to 510 c.c. perfusion fluid.
Recovered. Destroyed. Blood.
2
75 mg. strychnine sulfate added to 1200 c.c. perfusion fluid.
Recovered. Destroyed. Blood. Liver.
3
35 mg. strychnine sulfate added to 1350 c.c. perfusion fluid.
Recovered. Destroyed. Blood. Muscle.
4
50 mg. of strychnine injected into femoral vein.
Theoretical concentration. Blood. Muscle. Liver.
5
Minutes.
Hours.

Fig. 2. Almost no strychnine is shown to be disappearing from the perfusion fluid of a heart-lung preparation. The apparent slight decrease is almost within the biologic error inherent in the quantitative estimation of the drug. The first point charted for the concentration of strychnine in blood in all charts, represents the calculated concentration of the drug at the time it is injected.

Fig. 3. The relatively high concentration is shown of strychnine in the liver with the resultant precipitous fall in the blood content of the drug immediately following its addition to the circulation of a heart-lung-liver perfusion. Subsequently the strychnine largely disappears; only traces of it remain in either the blood or liver.

Fig. 4. Concentration is shown of strychnine in blood and muscle following the addition of 35 mgm. of the drug to the circulation of a heart-lung-leg preparation. The gradual accumulation of alkaloid in the muscles and the relatively small amount that is destroyed may be noted.

Fig. 5. The rapidity is shown with which strychnine accumulated in the liver of the intact animal after the intravenous injection of the drug, with the consequent precipitous fall in the amount of the alkaloid remaining in the circulating blood. Subsequently the hepatic content of strychnine was markedly decreased. The muscles retained a moderate share of strychnine at a rather constant level. The theoretic concentration is calculated on the basis that the drug diffuses equally through all the tissues of the animal.

inserted in the femoral artery and the leg is removed by amputation with disarticulation at the hip joint. The leg is placed at once in the warm container used for the liver, and perfusion through the femoral artery is started within seven to ten minutes after circulation in the leg is first interrupted. Any bleeding points on the cut surfaces of the muscles are clamped so that the return venous flow is almost entirely through the femoral vein. Specimens of blood and muscle are removed for analysis. Stimulation by faradic shocks of the sciatic nerve or individual muscles affords evidence of the functionating condition of the muscle during the course of the experiment. Four typical protocols follow.

Heart-lung perfusion. A dog, weighing 5 kgm., was used for the heart-lung preparation, which was completed according to the usual technic at 9:20 a.m. Five hundred cubic centimeters of defibrinated blood were placed in the venous reservoir. At 9:35 a.m., 30 mgm. of strychnine sulphate in 60 cc. of Ringer-Locke's solution were added to the perfusion fluid. The heart, which previously had been beating regularly, averaging 100 to 110 beats a minute, became slow (70 to 80), deliberate and irregular, until it failed at 10:35 a.m. The temperature of the blood varied from 38 to 39.5°C. Specimens of blood amounting to a total of 65 cc. were taken for analysis at 9:58, 10:22 and 10:35 a.m. (fig. 2).

Heart-lung-liver perfusion. A total of 1200 cc. of defibrinated blood was obtained from two large donors. A young dog, weighing 8.5 kgm., was used for the heart-lung circuit which was completed according to the usual technic at 10:14 a.m. Hepatectomy was then performed and cannulas were inserted into the hepatic artery and portal vein. The common bile duct was ligated. Perfusion of the liver was started at 10:25 a.m., eleven minutes after the normal circulation had been discontinued. Seventy-five milligrams of strychnine sulphate in 150 cc. of Ringer-Locke's solution were injected into the tube leading from the venous reservoir to the right auricle in a five-minute period from 10:34 to 10:39 a.m. During this interval the heart rate became slow (70 to 80) and irregular, but normal rate and rhythm returned almost immediately on completion of the injection. The experiment continued satisfactorily until 1:15 p.m., when the experiment was discontinued.

Specimens of blood amounting to 35, 80, 95, 100, 100, and 120 cc., respectively, were taken for analysis from the hepatic veins at 10:42, 10:49, 11:00, and 11:34 a.m. and at 12:05 and 1:15 p.m. Specimens from the liver, removed at 10:42 and 11:05 a.m. and 12:05 and 1:15 p.m. were taken from a different lobe in each instance. The flow of blood through the liver for the two hours and fifty minutes that the organ was perfused averaged 250 cc. a minute. The heart weighed 100 grams, and the liver weighed 324 grams (fig. 3).

Heart-lung-leg perfusion. The right hind leg of a dog weighing 16.5 kgm.

was shaved, cleansed, and dried before the establishment of the heart-lung circuit, which was completed at 8:32 a.m. Thirteen hundred fifty cubic centimeters of defibrinated blood were placed in the venous reservoir. A cannula was inserted into the right femoral artery, and the leg was amputated at the hip joint. It was placed in the warm container at once and perfusion through the femoral artery was started at 8:42 a.m. Prominent bleeding points on the cut surface of the leg were clamped, thus making the venous return almost entirely through the femoral vein. At 8:45 a.m., 35 mgm. of strychnine sulphate were mixed with the blood in the venous reservoir. The blood pressure was normal and the blood issuing from the heart was well oxygenated until the preparation was discontinued three hours after the strychnine was added.

The flow of blood through the leg, which was estimated whenever specimens of blood were taken, averaged 110 cc. a minute throughout the experiment. The blood returning from the leg was distinctly venous, in contrast to that which entered the femoral artery. Simultaneous specimens of blood and muscle were taken for analysis at 8:55, 9:25, 10:00, 10:55 and 11:45 a.m. The specimens of blood were taken from the femoral vein, and the specimens of muscle were removed from the deep tissues of the leg. At the conclusion of the experiment faradic stimulation of the sciatic nerve, as well as of individual muscles, caused marked contractions. The heart and leg weighed 200 and 1139 grams, respectively (fig. 4).

Results. Destruction of strychnine in the heart-lung circuit by itself is practically nil (fig. 2). The apparent disappearance of a small amount of the alkaloid can be almost entirely accounted for by the known loss of 10 to 15 per cent in the biologic estimation of the drug.

The heart-lung-liver perfusion affords definite evidence of the ability of the liver to destroy strychnine (fig. 3). The drug quickly leaves the perfusion fluid of such a preparation. The liver soon acquires a relatively large share of the strychnine, with the result that shortly after the drug is added to the circulating blood, the concentration of strychnine in the liver is far in excess of the concentration in the blood. Subsequently, the drug disappears from the blood rapidly and somewhat less quickly from the liver, with a simultaneous marked decrease in the total amount of strychnine that can be recovered. Inasmuch as the strychnine disappears from the liver without any coincident rise in the concentration of the drug in the blood, one would be led to assume that some alteration of the alkaloid actually occurs within the liver.

The heart-lung-leg perfusion (fig. 4), used as a control, shows that the tissues of the leg do not possess the power of the liver immediately to "arrest" a large amount of the circulating strychnine. Further evidence of this fact was afforded by the occurrence of the more prolonged cardiac

arhythmia and bradycardia when strychnine was added to the perfusion circuit in the absence of the liver. A certain amount of strychnine tends to accumulate in muscles so that the concentration of the drug in this tissue gradually was raised during the course of the experiment. This would seem to explain the gradual decrease of strychnine in the blood of the heart-lung-leg perfusion circuit. There is evidence that a small amount of the drug which is added cannot be recovered at the conclusion of the experiment. Since practically no strychnine is destroyed by the heart-lung circuit itself, it seems logical to assume that the tissues of the hind leg are capable of altering or destroying a small amount of strychnine.

In addition to the immediate arrest by the liver of a large proportion of the drug in the circulating fluid, the rapid destruction of strychnine which occurs in the liver during the course of perfusion, in contrast to that in the tissues of the perfused leg, demonstrates a specialization in the liver's ability to protect against this alkaloid.

Comment. By working rapidly, artificial perfusion of the liver can be started within ten minutes after the organ has been deprived of its normal circulation following ligation of the aorta. During this period the dog's own heparinized blood lies in the hepatic vessels, which obviates the undesirable practice of washing the liver with Locke's solution. The temporary occlusion of the portal vein and hepatic artery for a similar interval in the normal dog does not cause any obviously deleterious effect.

The lungs, besides affording normal aëration of the blood, are able to remove most of the vasoconstrictor toxin present in defibrinated blood before the liver is connected in the circuit. Blood enters the hepatic artery and the portal vein at the same pressure and with the same pulsations as in the intact animal. The rate of blood flow through the liver during experiments of this type averages 1.3 cc. each second for each 100 grams of liver. This is practically within the normal limits of the flow of blood through the dog's liver, which has been stated by Macleod to be 1.4 to 2.4 cc. each second for each 100 grams of liver.

There is considerable evidence to show that the liver is actually functioning during perfusion experiments of this type. The gross appearance of the organ, in the more successful experiments, is similar to that of the normal liver, as seen during laparotomy. Only at the end of a long experiment does slight edema occur. The blood leaving the hepatic veins is markedly venous in contrast to that which enters the liver. The appearance of a small amount of bile during the course of the perfusion, the cystic duct having been ligated, affords additional evidence of normal hepatic function.

One would not expect that a perfused organ possesses 100 per cent of its normal efficiency. It would seem, therefore, that positive evidence of the ability of the liver to destroy strychnine during perfusion would

afford only a minimal estimate of the actual extent of this power as it exists in the intact animal. It appears reasonable to assume that the decrease of this function in the surviving organ would be relatively greater for the more highly specialized tissue. Consequently, one might anticipate an even greater difference than was shown in these experiments in the ability of muscle and hepatic tissue to destroy strychnine in the living animal.

SUSCEPTIBILITY OF NORMAL AND DEHEPATIZED DOGS TO STRYCHNINE. The dehepatized animal in many respects offers an ideal object for the study of the detoxicating function of the liver if certain factors are kept in mind. The dehepatized dogs used in these experiments were prepared according to the method of Markowitz and Soskin, with the modification that the portal vein and vena cava were completely ligated at a second operation, several weeks before the liver was finally removed. This additional operation assured adequate collateral circulation when hepatectomy was performed.

Method of procedure. The "just convulsive" intravenous dose of strychnine for each animal was determined, prior to any surgical procedure. When the dog had recovered from the immediate operative effects of hepatectomy, the same amount of the drug was again administered in the same manner and with the same speed. Any variation in the reaction caused by the drug before and after removal of the liver was thus apparent. After the liver was removed, 0.5 gram of glucose for each kilogram of body weight was given hourly in order to maintain a normal level of blood sugar. Dogs on whom ordinary laparotomy had been performed served as controls. A 0.04 per cent aqueous solution of strychnine was used throughout. All the injections of strychnine were made directly into the femoral vein.

Results. The dehepatized dog was found to be far more susceptible to strychnine than the intact animal. Half of the previously determined "just convulsive" dose was sufficient to cause convulsions in the dehepatized animal. Furthermore, two-thirds of this dose caused severe convulsions, which reappeared on stimulation for several hours and sometimes terminated fatally.

Comment. That the effect of operation, per se, should influence the dog's susceptibility to strychnine to such an extent seems unlikely, since following other operations more prolonged than that of the removal of the liver (reverse Eck fistula), the toxicity of a given dose of strychnine was practically unaltered. After the liver had been removed, the dogs were all walking around normally at the time when strychnine was given, and they did not appear more incapacitated than any dog a few hours after laparotomy of average severity. By determining the susceptibility to strychnine of each animal before and after dehepatization, any errors

caused by individual variations among the dogs were obviated. Since, in a carefully controlled series of experiments of this type, the dehepatized dog was found to be distinctly more susceptible to strychnine than the intact animal, it seems justifiable to assume that the liver normally exercises a protective function against strychnine.

RATE OF DISAPPEARANCE OF STRYCHNINE IN NORMAL AND DEHEPATIZED DOGS. The dehepatized dogs used in these experiments were prepared according to the three-stage technic of Mann (6). In order to establish a basis for comparison of the disappearance of strychnine, observations were first made on the normal dog. Frequent determinations of the concentration of strychnine in blood, liver and muscles were made, following a large intravenous injection of the drug. The studies were made on the etherized animal.

Method. After the animal was etherized, a cannula was placed in the right femoral artery and the femoral vein was cleared. In the normal dogs, the liver was exposed. Approximately 6 mgm. of strychnine for each kilogram of body weight, in a solution containing 0.5 mgm. for each cubic centimeter, were injected into the femoral vein over a period of five minutes.

Specimens of blood were taken from the femoral artery a few minutes after the injection and subsequently at varying intervals. Transfusions of heparinized blood were given through the femoral vein as indicated, in order to compensate for the removal of the necessarily large specimens of blood. Specimens of liver, in normal dogs, were taken from the left lateral lobes. Specimens of muscle were removed from the left gluteal region. Care was taken in removing the specimens of the liver and muscle to avoid any tissue which might have impaired circulation because of previous excisions. An adequate amount of glucose was given to the dog during the course of the experiment. Two typical protocols follow.

Normal animal. A young dog, weighing 8.5 kgm., was etherized in the usual manner at 9:20 a.m., after which intratracheal etherization was instituted. Between 9:25 and 9:29 a.m., after a cannula had been inserted in the femoral artery and the femoral vein and the liver had been exposed, 50 mgm. of strychnine sulphate in 100 cc. of Ringer-Locke's solution were injected into the femoral vein. During the injection the heart became slow and irregular, but normal rate and rhythm had returned by 9:30 a.m. The liver weighed 308 grams. Specimens of blood, muscle, and liver were taken for analysis as indicated in figure 5.

Dehepatized animal. Hepatectomy was completed at 9:15 a.m. on a dog weighing 13.5 kgm. The postoperative condition of the animal was excellent. He was given 30 cc. of a solution of 25 per cent glucose intravenously at 10:30 a.m., and 12:30, 1:50 and 3:27 p.m.

The experiment was started with etherization at 2:30 p.m. Forty

milligrams of strychnine sulphate in 100 cc. of physiologic sodium chloride solution were injected into the femoral vein between 2:19 and 2:26 p.m. Cardiac action was embarrassed for several minutes afterward. Specimens of blood and muscle were removed as in the previous experiment. Transfusions of blood were given when indicated (fig. 6).

Results. Strychnine disappears rapidly from the blood stream of the intact animal following the intravenous injection of the drug. The precipitous fall in the level of strychnine in the blood appears to be explained by the immediate accumulation of a large quantity of the alkaloid within the liver (fig. 5). Four minutes after completion of an intravenous injection of 50 mgm. of strychnine in the normal dog, the concentration

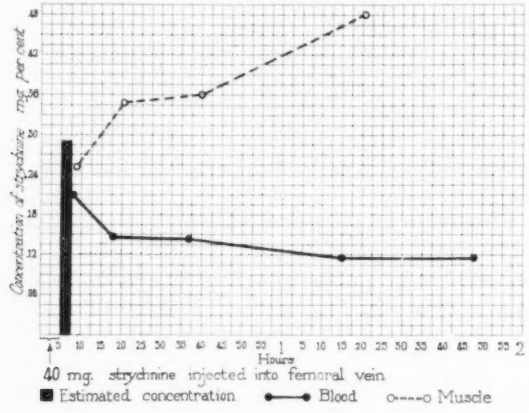

Fig. 6. The relatively slight decrease is shown of strychnine in the blood after intravenous injection of the drug in the dehepatized animal with the simultaneous accumulation of the alkaloid in muscle.

of the drug in the liver was more than seven times greater than that in the blood. Subsequently the content of strychnine in the liver was greatly reduced, without any simultaneous rise in the concentration of the drug in the blood, which leads one to believe that destruction of the alkaloid occurs actually within the liver. The muscles retained a rather constant amount of strychnine, somewhat above the level in the blood, and did not show direct evidence of destruction of strychnine in situ.

An entirely different picture is presented by the dehepatized dog. In this animal (fig. 6) an intravenous injection of strychnine was followed by a gradual and relatively slight fall in the concentration of strychnine in the blood. The rather rapid accumulation that occurs within the muscles appears sufficient to account for moderate decrease in the blood. Here again, there is no direct evidence that muscle is capable of destroying

strychnine. In brief, it seems that the liver is a most important organ, not only in the rapid removal of strychnine from the blood, but also in the subsequent destruction of the strychnine.

Comment. Anesthetized animals were used in this study since it was found that ten to fifteen times the ordinarily fatal dose of strychnine could be given to the deeply etherized dog with impunity, provided intratracheal artifical respiration was employed. A similar power, in combating the action of strychnine, has been ascribed to chloral hydrate, as well as other sedatives. Deep anesthesia is essential, as otherwise convulsions will ensue when large amounts of strychnine are given. If convulsions occur in the dehepatized dog, more than the usual amount of glucose must be supplied because of the intense muscular activity.

The effects of ether anesthesia on the function of the liver are realized (7). One would expect, therefore, an even greater destruction of strychnine by the liver of the unanesthetized animal. Without etherization, however, quantitative studies on the rate of disappearance of strychnine from the body would be extremely difficult because of the minute quantity of the drug that would be present in even large amounts of blood and tissues, following an ordinary sublethal dose of strychnine. In fact it would be necessary to exsanguinate the animal for a single specimen of blood. The use of small dogs facilitates these experiments, since smaller replacement transfusions suffice. This dilution of the dog's own blood with blood free from strychnine is a constant factor in the experiments on both normal and dehepatized animals.

CONCLUSIONS

1. Of the six methods employed for investigating the detoxicating function of the liver, the study of the dehepatized dog and the perfused liver appears to have yielded the most conclusive and accurate data. The exclusive use of any single method is not recommended. However, by the combined use of the analytic and the synthetic methods (organism without a liver and surviving liver without the organism), respectively, definite evidence concerning the liver as a detoxicating organ can be obtained.

2. Starling's heart-lung preparation can be used for the perfusion of the liver, or the hind limb of a dog. By this means it is possible to investigate the ability of the liver and of the hind limb to destroy any substance for which a suitable quantitative method is available.

3. The properly prepared dehepatized dog is an ideal subject for the study of the detoxicating function of the liver.

4. The liver of the dog apparently possesses a highly specialized ability to immediately arrest and subsequently destroy strychnine.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XXI. The Rôle of the Liver in the Formation of Lymph

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Because the liver is large, very active, and rich in lymphatic drainage, it has been rightly suspected of contributing in large measure to the lymph that finally empties from the thoracic duct. Although Starling observed that ligating the lymphatic trunks in the portal fissure did not abolish the lymphagogic effect of injected peptone, nevertheless he concluded that the liver played an important part in the formation of lymph. His conclusion was based chiefly on experiments as follows: Obstruction of the inferior vena cava above the diaphragm causes marked increase in the flow of lymph only when the periportal lymphatics, through which the major portion of the lymph formed in the liver passes to the thoracic duct, are intact. When these were ligated, the flow of lymph from the thoracic duct was not increased by the same procedure. Based almost exclusively on such experimentation, the opinion has been current that the liver plays a major part in the formation of the lymph that can be collected from the thoracic duct.

In order to obtain some information regarding the rôle of the liver in the formation of lymph, we carried out experiments in which lymphatic flow from the thoracic duct was observed before and after ligation of the periportal lymphatics and before and after total removal of the liver.

Methods. Under ether anesthesia a cannula was inserted into the thoracic duct of healthy dogs. Two hours previous to the operation the animals were given a liberal quantity of water by stomach tube; otherwise they were in a fasting condition. Usually there was no difficulty in identifying, and inserting the cannula into, the thoracic duct. During observations the animals were maintained under uniform ether anesthesia. The lymph was collected into a graduated centrifuge test tube; when possible this was changed every ten minutes so as to obtain a curve of the ten-minute flow of lymph.

Results. Ligating the periportal lymphatics did not cause any change except temporary increase of the flow of lymph which can be attributed to

handling the intestines and other operative manipulations (fig. 1, dogs 1 and 2).

In a certain number of experiments a solution of Witte's peptone was injected intravenously. The dosage was usually 3 cc. of a 5 per cent solution for each kilogram of body weight. Again no effect was produced in the flow of lymph from the thoracic duct by ligating the periportal lympha-

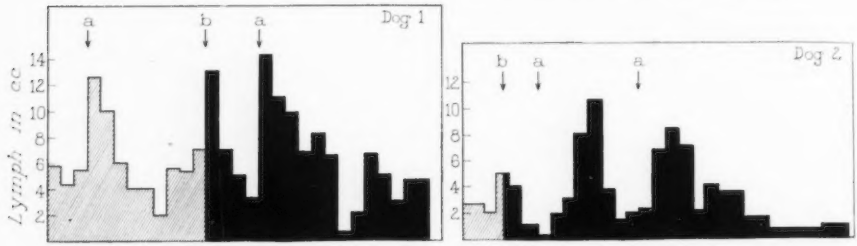

Fig. 1. *a*, Intravenous injection of 5 per cent solution of peptone, 3.0 cc. for each kilogram of body weight; *b*, ligation and destruction of portal lymphatics completed; *c*, removal of liver completed, and *d*, intravenous injection of 50 per cent solution of glucose, 2.3 cc. for each kilogram of body weight.

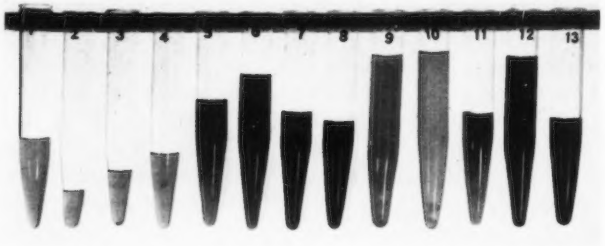

Fig. 2. Tubes 1 to 13 show the lymph collected from the thoracic duct in consecutive ten-minute periods. Tubes 1 to 4, normal flow; *a*, removal of the liver; tubes 5 to 8, response to removal of liver; *b*, injection of 3 cc. of 5 per cent solution of peptone for each kilogram of body weight; tubes 9 and 10, lymphagogic response to peptone is shown; the animal was hypoglycemic; *c*, flow of lymph ceases; injection of 2.3 cc. of 50 per cent solution of glucose for each kilogram of body weight; tubes 11 to 13, response to injection of glucose.

tics either before the peptone was injected or afterward. In both instances the lymphagogic action was as marked as would be expected in the normal animal (fig. 1, dogs 1 and 2).

In another set of experiments a cannula was inserted into the thoracic duct as usual in an animal that was suitably prepared for removal of the liver. The flow of lymph was observed for thirty minutes after which the

abdomen was opened and the liver was removed. The results of these experiments were conclusive (fig. 1 and fig. 2). As noted in the representative protocol, the act of removing the liver was accompanied by an increased flow of lymph from the thoracic duct. The incision in the abdomen was closed in the usual manner, but in spite of the fact that operative

TABLE 1

*Observations on the flow of lymph in a dog, weighing 17.5 kgm., under ether anesthesia**

SAMPLE	A.M.	AMOUNT	RATE EACH TEN MINUTES	COMMENT
		cc.		
1	9:20– 9:30	4.6	4.6	Lymph clear
2	9:30– 9:40	1.4	1.4	Prompt clotting
3	9:40– 9:50	2.0	2.0	Prompt clotting
4	9:50–10:00	3.4	3.4	
	9:52 Removal of liver started			
5	10:00–10:10	8.0	8.0	Lymph red; considerable blood in it
	10:06 Incision closed			
6	10:10–10:20	11.0	11.0	Much blood in lymph
7	10:20–10:30	6.5	6.5	Much blood in lymph
8	10:30–10:40	6.0	6.0	Much blood in lymph
9	10:40–10:45	5.0		
	10:43 3 cc. of 5 per cent solution of peptone for each kilogram of body weight injected into femoral vein			
	10:45–10:48	9.0	17.5	
10	10:48–10:53	8.0		
	10:53–10:58	5.9	13.9	
	10:58–11:00	2.5		
11	11:00 Shallow breathing; 40 cc. 50 per cent glucose given intravenously			
	11:00–11:08	4.3	6.8	More blood in lymph than at any previous time
12	11:08–11:13	5.7	12.0	
	11:13–11:18	6.3	12.6	
13	11:18–11:28	7.0	7.0	All lymph clotted promptly
	4 cc. of 5 per cent solution of peptone for each kilogram of body weight given. Dog manifested severe shock and succumbed in spite of the injection of more glucose			

* Free blood or fluid was not present either in the wound or in the abdominal cavity. The viscera were congested as is always the case, in shock from peptone.

manipulation had ceased, flow of lymph continued. The injection of peptone immediately called forth a marked increase in the flow of lymph. About twenty minutes after the injection of peptone, some of the symptoms of hypoglycemia developed and the flow of lymph almost stopped. The intravenous injection of a solution of 50 per cent glucose (2.3 cc. for each kilogram of body weight) restored the animal and the flow of lymph increased (tabulation).

COMMENT. There is reason to believe that an organ produces more lymph in a state of activity than at rest. The statement is made that the lymph flows from the lymphatics of the posterior extremity of a dog during faradization of the sciatic nerve when there is no flow of lymph from the opposite inactive leg. It would be supposed, therefore, that an organ such as the liver which gives so many evidences of continuous activity would form a considerable quantity of lymph. Such a belief, however, is by no means identical with the thesis that the liver plays a major part in the production of lymph when compared with what is produced by the remainder of the organism. In our experiments the removal of the liver did not diminish the flow of lymph from the thoracic duct. We are not prepared to state that hepatectomy increases this flow, since the increase may be incidental to handling the intestines and to a slight amount of back-pressure in the portal area.

That the injected peptone resulted in its usual lymphagogic action indicates again that the extra lymph formed under the influence of peptone need not be attributed to the liver.

SUMMARY

The flow of lymph from the thoracic duct was observed before and after ligation of the periportal lymphatics without any difference being detected.

The lymphagogic action of peptone was investigated in normal etherized dogs before and after ligation of the periportal lymphatics. Differences were not observed.

Removal of the liver by the usual technic did not reveal diminution in the flow of lymph.

The intravenous injection of peptone into a dehepatized dog was followed by the usual lymphagogic effect.

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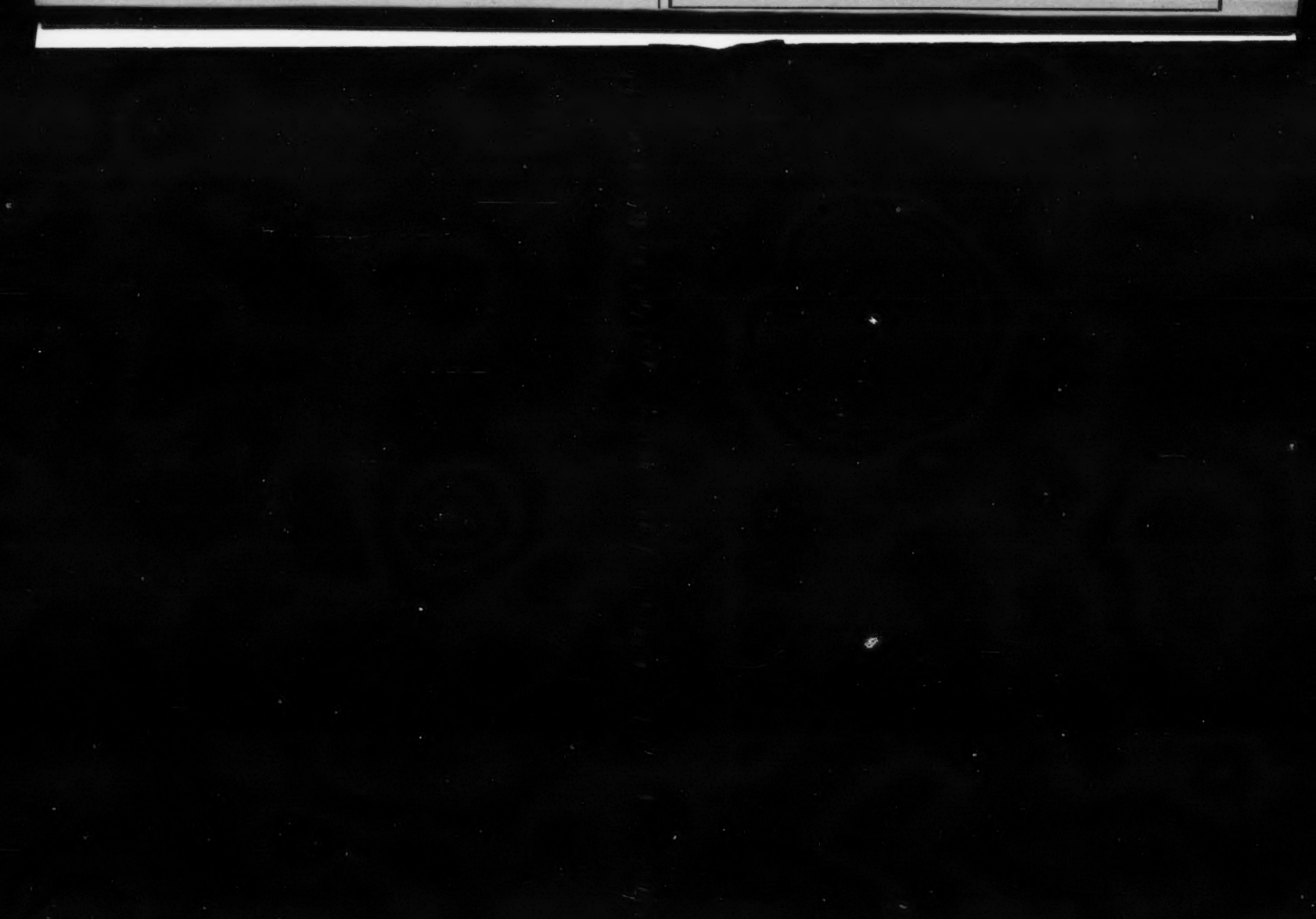

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